

Science



21 May 2010 | \$10

EDITORIAL

- 953 Banning Nuclear Tests
Pierce S. Corden

NEWS OF THE WEEK

- 958 Synthetic Genome Brings New Life to Bacterium
 >> *Science Express Research Article*
 by D. G. Gibson et al.
- 959 Will Britain's Coalition Wield the Funding Ax?
- 960 From Science's Online Daily News Site
- 960 Former NIH Director Varmus to Head Cancer Institute
- 960 House Blocks Bill to Boost Research Spending
- 961 From the Science Policy Blog
- 962 Five Questions on the Spill

NEWS FOCUS

- 964 Battle of the Titans
- 969 Animal Communication Helps Reveal Roots of Language
 >> *Science Podcast*

LETTERS

- 973 Cretaceous Extinctions: Multiple Causes
 J. D. Archibald et al.
- Cretaceous Extinctions:
 The Volcanic Hypothesis
 V. Courtillot and F. Fluteau
- Cretaceous Extinctions:
 Evidence Overlooked
 G. Keller et al.
- Response
 P. Schulte et al.
- Honing the Test-and-Treat HIV Strategy
 T. D. Mastro and M. S. Cohen
- 974 CORRECTIONS AND CLARIFICATIONS

BOOKS ET AL.

- 977 The Calculus of Selfishness
 K. Sigmund, reviewed by D. Krakauer
- 978 Identity Economics
 G. A. Akerlof and R. E. Kranton,
 reviewed by R. Sugden

POLICY FORUM

- 979 The Smart Electricity Grid and Scientific Research
 J. Beyea

PERSPECTIVES

- 981 *Salmonella's* Safety Catch
 R. J. Collier
 >> Report p. 1040
- 982 The Enigmatic Inner Core
 B. A. Buffett
 >> Reports pp. 1014 and 1018
- 983 Signaling Through Cooperation
 E. D. Levy et al.
 >> Report p. 1043
- 985 Following the Motions of Water Molecules in Aqueous Solutions
 J. L. Skinner
 >> Reports pp. 1003 and 1006
- 986 Weight Loss with Magnesium Alloys
 T. M. Pollock
- 987 Stochastic Emergence of Groupthink
 A. Prindle and J. Hasty
 >> Report p. 1021

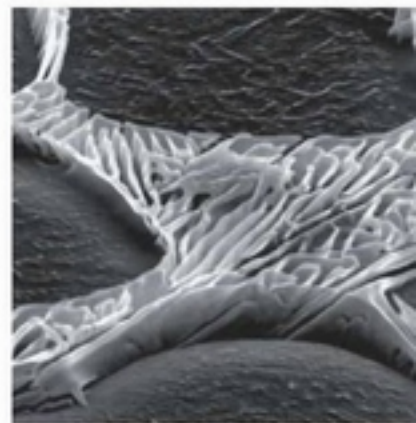
REVIEW

- 989 Primordial Gravitational Waves and Cosmology
 L. M. Krauss et al.

CONTENTS continued >>



page 969



page 986



COVER

Tumor-penetrating transport of cancer drugs is activated by the peptide iRGD (multicolored ring), which binds to integrin receptors (blue and yellow) in tumor blood vessels, and is subsequently cleaved. The cleaved peptide then binds to neuropilin-1 receptors (purple), activating a transport system that carries coadministered drugs, such as antibodies (green), deeper into tumor tissue. See page 1031.

Image: Peter Allen/University of California, Santa Barbara

DEPARTMENTS

- 949 This Week in Science
- 955 Editors' Choice
- 956 Science Staff
- 957 Random Samples
- 1047 New Products
- 1048 Science Careers

BREVIA

- 993 **Adaptive Evolution of an sRNA That Controls *Myxococcus* Development**
Y.-J. N. Yu et al.
Mutation of a small noncoding RNA drives adaptive evolution in a social bacterium.

RESEARCH ARTICLE

- 994 **A Catalog of Reference Genomes from the Human Microbiome**
The Human Microbiome Jumpstart Reference Strains Consortium
Standardized protocols and methods are being established for large-scale sequencing of the microorganisms living on humans.

REPORTS

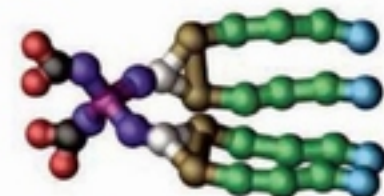
- 999 **Observation of Plasmarons in Quasi-Freestanding Doped Graphene**
A. Bostwick et al.
Doping of graphene introduces two new crossing points of the conduction and valence-electron bands.
- 1003 **Large Angular Jump Mechanism Observed for Hydrogen Bond Exchange in Aqueous Perchlorate Solution**
M. Ji et al.
Water molecules shift orientation between dissolved ions and the surrounding liquid by taking large, sudden steps.
- 1006 **Cooperativity in Ion Hydration**
K. J. Tielrooij et al.
When salts dissolve in water, the separated cations and anions can still collectively impact the liquid structure.
>> Perspective p. 985
- 1009 **Self-Assembly of Janus Dendrimers into Uniform Dendrimersomes and Other Complex Architectures**
V. Percec et al.
Amphiphilic, spherically shaped polymers self-assemble into larger hollow complexes that could be used for drug delivery.
- 1014 **Lopsided Growth of Earth's Inner Core**
M. Monnereau et al.
The asymmetry of the inner core is explained by iron crystallization on one side and melting on the other.
- 1018 **Regional Variation of Inner Core Anisotropy from Seismic Normal Mode Observations**
A. Deuss et al.
Seismic data from the inner core reveal that anisotropic regions overlap with gravitational anomalies.
>> Perspective p. 982

- 1021 **The Onset of Collective Behavior in Social Amoebae**
I. Gregor et al.
Stochastic pulsing of individual cells plays a critical role in initiating cyclic adenosine monophosphate pulses.
>> Perspective p. 987
- 1025 **Structural Insights into the Assembly and Function of the SAGA Deubiquitinating Module**
N. L. Samara et al.
Structures give insight into how a regulator of eukaryotic gene expression achieves one of its chromatin-modifying functions.
- 1029 **Network Diversity and Economic Development**
N. Eagle et al.
Social diversity is associated with economic development.
- 1031 **Coadministration of a Tumor-Penetrating Peptide Enhances the Efficacy of Cancer Drugs**
K. N. Sugahara et al.
Anticancer drugs are more effective in mice when they are injected with a peptide that helps the drugs penetrate the tumor.
- 1036 **Five-Vertebrate ChIP-seq Reveals the Evolutionary Dynamics of Transcription Factor Binding**
D. Schmidt et al.
Binding of two liver-specific transcription factors in several vertebrate species reveals complex regulatory evolution.
- 1040 **pH Sensing by Intracellular *Salmonella* Induces Effector Translocation**
X.-J. Yu et al.
Intravacuolar *Salmonella* sense host cytosolic pH, resulting in degradation of a regulatory complex and effector translocation.
>> Perspective p. 981
- 1043 **A Global Protein Kinase and Phosphatase Interaction Network in Yeast**
A. Breitkreutz et al.
Phosphorylation reactions in budding yeast reveal the regulatory architecture of a fundamental cellular control system.
>> Perspective p. 983

CONTENTS continued >>



pages 987 & 1021



page 1009



page 1029

SCIENCEONLINE

SCIENCEEXPRESS

www.sciencexpress.org

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

D. G. Gibson et al.

A synthetic *Mycoplasma mycoides* genome transplanted into *M. capricolum* was able to control the host cell.

10.1126/science.1190719

>> News story p. 958; Science Podcast

ATP-Binding Cassette Transporters and HDL Suppress Hematopoietic Stem Cell Proliferation

L. Yuan-Chen et al.

Pathways that reduce cholesterol in atherosclerosis also suppress increased immune cell numbers associated with the disease.

10.1126/science.1189731

Covering a Broad Dynamic Range: Information Processing at the Erythropoietin Receptor

V. Becker et al.

Modeling and experiments help to explain responsiveness of red blood cell precursors to very large changes in a proliferative signal.

10.1126/science.1184913

Identification of Germline Stem Cells in the Ovary of the Teleost Medaka

S. Nakamura et al.

Ongoing follicle production is maintained by stem cells in an adult fish ovary.

10.1126/science.1185473

Measurement of the Instantaneous Velocity of a Brownian Particle

T. Li et al.

An optically trapped silica bead in solution is used to probe assumptions underlying statistical theories of Brownian motion.

10.1126/science.1189403

>> Science Podcast

Closing Loopholes: Getting Illegal Fishing Under Control

S. Flothmann et al.

10.1126/science.1190245

SCIENCENOW

www.sciencenow.org

Highlights From Our Daily News Coverage

Golden Years Truly Are Golden

Massive phone survey reveals that people are happier after age 50.

The Scent That Makes Mice Run Scared

Mice are hard-wired to detect urinary proteins secreted by a wide variety of predators.

Vision Cells Help Set the Body's Clock

Research could lead to new therapies for depression and insomnia.

SCIENCE SIGNALING

www.sciencesignaling.org

The Signal Transduction Knowledge Environment

RESEARCH ARTICLE: TREM2- and DAP12-Dependent Activation of PI3K Requires DAP10 and Is Inhibited by SHIP1

Q. Peng et al.

The inositol phosphatase SHIP1 binds to a receptor-adaptor complex on osteoclasts to prevent recruitment of PI3K and inhibit receptor signaling.

RESEARCH ARTICLE: c-mip Impairs Podocyte Proximal Signaling and Induces Heavy Proteinuria

S. Zhang et al.

Overexpression of the protein c-mip in mice produces phenotypes similar to various idiopathic kidney disorders.

PERSPECTIVE: Gyrate—CCM3 Dances with a Different Angiogenic Partner

L. A. Dyer et al.

CCM3 is a key regulator of a major signaling pathway involved in vascular development.

SCIENCE CAREERS

www.sciencereers.org/career_magazine

Free Career Resources for Scientists

Hard Work and Drive Propels a Scientist From China to the United States

R. Mejia

A passion for learning led virologist Fenyong Liu into top American labs.

Tooling Up: The Medical Writing and Corporate Intelligence Career Tracks

D. Jensen

Jim Gardner follows a career as a medical writer with another as a corporate sleuth.

GrantsNet Funding News

D. Adams

Learn about the latest research and training grants for students, postdocs, and faculty.

SCIENCE TRANSLATIONAL MEDICINE

www.sciencetranslationalmedicine.org

Integrating Medicine and Science

EDITORIAL: On Board with the Cures Acceleration Network

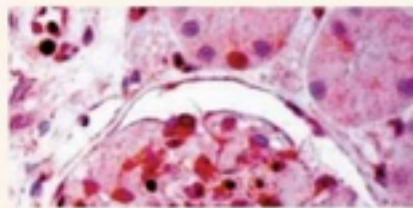
E. G. Nobel

The new U.S. health care legislation includes funding mechanisms to accelerate the development of "high-need cures."

REVIEW: Deciphering Genetic Disease in the Genomic Era—The Model of GNRH Deficiency

G. P. Sykiotis et al.

Elucidation of the genetic causes of human disease requires unbiased, patient-oriented investigations.



SCIENCE SIGNALING

New target for kidney disease.

RESEARCH ARTICLE: Androgen Receptor Promotes Hepatitis B Virus-Induced Hepatocarcinogenesis Through Modulation of Hepatitis B Virus RNA Transcription

M.-H. Wu et al.

Targeting the androgen receptor may prevent hepatitis B virus-induced liver cancer.

RESEARCH ARTICLE: Tryptophan Catabolism by Indoleamine 2,3-Dioxygenase 1 Alters the Balance of T_H17 to Regulatory T Cells in HIV Disease

D. Favre et al.

PERSPECTIVE: Insights into Therapy—Tryptophan Oxidation and HIV Infection

M. F. Murray

New findings link tryptophan breakdown to immune cell imbalance and chronic inflammation associated with HIV/AIDS.

SCIENCEPODCAST

www.sciencemag.org/multimedia/podcast

Free Weekly Show

Download the 21 May Science Podcast to hear about bacterial cells controlled by a synthetic genome, instantaneous Brownian velocity, the evolution of language, and more.

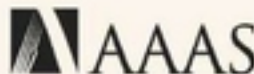
SCIENCEINSIDER

news.sciencemag.org/scienceinsider

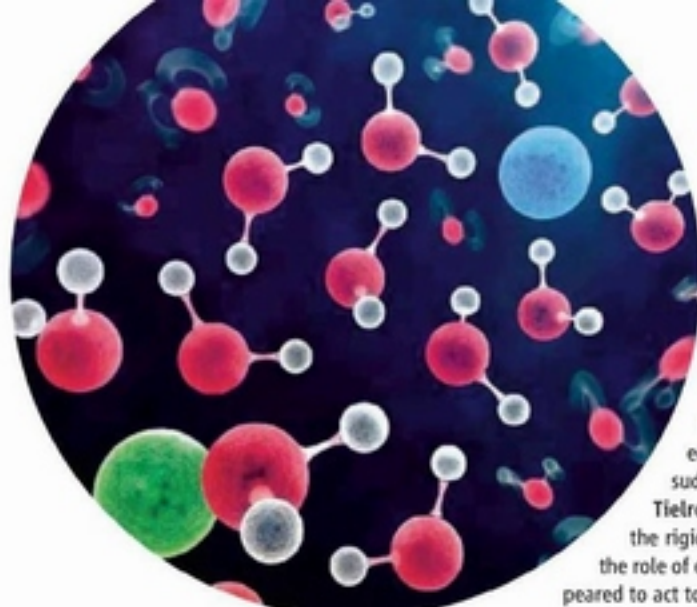
Science Policy News and Analysis

SCIENCE (ISSN 0036-8073) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2010 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$146 (124 allocated to subscription). Domestic institutional subscription (51 issues): \$1912. Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request. GST #R1234 88123. Publications Mail Agreement Number 2069624. Printed in the U.S.A.

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. Postmaster: Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-4378. Single-copy sales: \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$10.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for Science is 0036-8073. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.



ADVANCING SCIENCE. SERVING SOCIETY



<< Wet Twists and Turns

When salts dissolve in water, their constituent positively and negatively charged ions are pulled apart and surrounded by shells of H_2O molecules (see the Perspective by Skinner). Ji *et al.* (p. 1003) looked closely at the motion in these shells, using a type of vibrational spectroscopy sensitive to both the orientation and to the neighbors of the targeted molecules. In agreement with recent theoretical predictions, the individual water molecules shifted orientation between an anion and the surrounding liquid in sudden discrete steps, rather than by making smooth incremental rotations. Tielrooij *et al.* (p. 1006) compared the relative impacts of cations and anions on the rigidity of the wider water network, using spectroscopic techniques sensitive to the role of each ion. Certain cation/anion combinations, such as magnesium sulfate, appeared to act together to restrict water motion beyond the boundaries of individual shells.

Astronomical Inflation

In astronomy, according to the theory of inflation, the universe underwent a period of extremely accelerated expansion when it was only a fraction of a second old. This process made the universe flat, isotropic, and homogeneous, and it explains how quantum mechanical seeds developed into the large-scale structure we observe today. The theory also predicts that gravitational waves were produced during the early inflationary phase. Alternative theories either predict no gravitational waves or waves with different properties. Krauss *et al.* (p. 989) review how primordial gravitational waves could provide information on the physics shortly after the Big Bang and how they could be detected indirectly through their imprint on the cosmic microwave background radiation—relic radiation from the Big Bang released when the universe became transparent to electromagnetic radiation.

All Together Now

In the social amoeba *Dictyostelium discoideum*, periodic synthesis and release of cyclic AMP (cAMP) guides the cellular aggregation required to form fruiting bodies. It has been unclear whether the initiation of this behavior is owing to synchronization of autonomously oscillating cells or whether individual cells remain nonoscillatory unless the entire population becomes oscillatory. Gregor *et al.* (p. 1021, published online 22 April; see the Perspective by Prindle and Hasty) used live-cell imaging to show that cAMP pulses originate from a specific location in space and that individual cells move in and out of these signaling centers. The observations suggest that oscillations do not originate from autonomous activities of specialized cells. However, individual cells do display stochastic cAMP-pulsing below a threshold external concentration of cAMP, and the generation of synchronized oscillations could only

be modeled accurately when this random pulsing was taken into account.

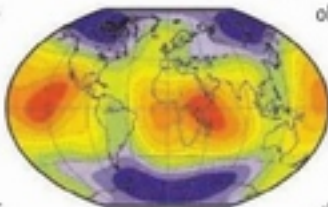
Clearing Up the Inner Core

The behavior of Earth's core controls the planet's heat budget and magnetic field, yet its structure remains enigmatic. For instance, the seismic properties of the solid inner core suggest hemispherical structural asymmetry, but questions remain as to how these variations arose (see the Perspective by Buffett). Monnerieu *et al.* (p. 1014, published online 15 April) modeled grain sizes of crystalline iron—the predicted dominant mineral phase in the core—and found that a slow translational motion eastward may trigger melting in the Eastern Hemisphere and solidification in the Western Hemisphere, creating a lopsided core. Deuss *et al.* (p. 1018, published online 15 April) examined the normal-mode seismic structure of the inner core, collected from 90 large earthquakes, which reveal not just simple hemispherical variations, but more nuanced regional structures. The overlap of the seismic data with Earth's magnetic field suggests that directionally dependent crystal alignment in the inner core formed during the solidification of the core or as a consequence of strong forces exerted by magnetism.

More Crossings for Graphene

Graphene, which consists of single sheets of graphite, has a number of distinctive electronic

properties, including a conical structure that leads to a "Dirac point" where the valence and conduction band intersect at a zero-energy point. Bostwick *et al.* (p. 999) used angle-resolved photoemission spectroscopy to study graphene that was doped with alkali atoms and suspended from its substrate. They observed features associated with plasmarons, which arise from the interaction of the charge carriers with plasmons, the density oscillations of the electron gas. The Dirac crossing now becomes three crossings: one that involves charge bands, one involving plasmarons, and one involving the interaction between the two.



Janus Drug Delivery Vehicle

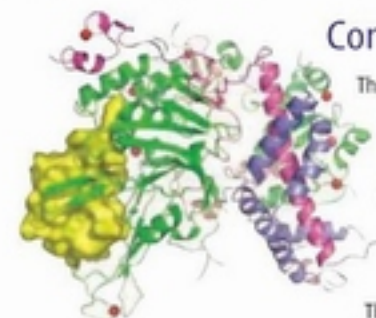
Efficient drug delivery vehicles need to be produced in a limited size range and with uniform size distribution. The self-assembly of traditional small-molecule and polymeric amphiphiles has led to the production of micelles, liposomes, polymeric micelles, and polymersomes for use in drug delivery applications. Now, Percic *et al.* (p. 1009) describe the self-assembly of Janus-type (i.e., two-headed) dendrimers to produce monodisperse supramolecular constructs, termed "dendrimerosomes," and other complex architectures. The structures, which showed long-term stability as well as very narrow size distributions, were easily produced by the injection of an ethanolic solution of the dendrimer into water. The dendrimerosomes could be loaded with the anticancer drug doxorubicin and exhibited controlled drug release with changing pH.

Continued on page 951

Continued from page 949

Network for Recovery

A long-standing theory suggests that social diversity leads to economic development. By combining the United Kingdom's telephone communication records (both landline and mobile) with information on regional economic conditions, **Eagle et al.** (p. 1029) demonstrate that network diversity alone accounts for over three-quarters of the variance of a region's economic status. Although the data cannot be used to show causality, the association suggests that economic development and recovery may depend not solely on monetary stimulus but also on the development of a nation's social infrastructure.



Complex SAGA

The SAGA (Spt-Ada-Gcn5-Acetyltransferase) complex, which is conserved in eukaryotes, plays a key role in regulating gene expression. It is comprised of 21 proteins, and its functions include histone acetylation and deubiquitination. **Samara et al.** (p. 1025, published online 15 April) now report the structure of the SAGA deubiquitinating module (DUBm), a four-protein subcomplex, both on its own and bound to ubiquitin aldehyde.

The domains are interconnected and stabilized by eight zinc atoms. The organization gives insight into why DUBm complex formation is required to activate the catalytic domain of the enzyme and suggests how DUBm might bind to monoubiquitinated histones.

Penetrating Attack on Tumors

While considerable research effort in oncology is focused on the design of new cancer drugs, an important but relatively understudied research area is the development of methods that optimize the delivery and tumor penetration of existing cancer drugs. Previous work has characterized a peptide (iRGD) that selectively targets and penetrates tumor tissue by virtue of its specific interaction with tumor blood vessels. Now, studying mouse models, **Sugahara et al.** (p. 1031, see the cover) show that coinjection of the iRGD peptide increases the tumor penetration and antitumor activity of several cancer drugs, including the cytotoxic agent doxorubicin and the therapeutic antibody trastuzumab (Herceptin), without increasing their harmful effects on healthy tissue. Importantly, these effects did not require chemical conjugation of the cancer drugs to the peptide.

Cytosol, *Salmonella*, and pH

Salmonella and other bacterial pathogens grow inside animal host cells within intracellular vacuoles. The bacteria secrete effector proteins across the vacuole membrane, altering the host-cell physiology to the pathogen's advantage. The secretion process involves a specialized secretory apparatus, the type III secretion system, whose assembly is triggered by the low pH within the host-cell vacuole. Now, **Yu et al.** (p. 1040, published online 15 April; see the Perspective by **Collier**) have identified neutral pH as a physiological signal for effector translocation by intracellular *Salmonella*. The process involves the disassembly of a membrane-bound regulatory complex that is also found in other animal pathogens. Thus, *Salmonella* exploits the low pH of the vacuole as a signal to induce assembly of the secretion system, and then the neutral pH of the cytoplasm to trigger effector translocation.

Budding Yeast Kinome Revealed

Covalent modification of proteins by phosphorylation is a primary means by which cells control the biochemical activities and functions of proteins. To better understand the full spectrum of cellular control mechanisms mediated by phosphorylation, **Breitkreutz et al.** (p. 1043; see the Perspective by **Levy et al.**) used mass spectrometry to identify proteins that interacted with the complete set of protein kinases from budding yeast and with other molecules, including phosphatases, which influence phosphorylation reactions. The results reveal a network of interacting protein kinases and phosphatases, and analysis of other interacting proteins suggests previously undiscovered roles for many of these enzymes.



Pierce S. Corden is a visiting scholar at the AAAS Center for Science, Technology, and Security Policy in Washington, DC, and has worked on nuclear testing issues since 1971. E-mail: pcorden@aaas.org

Banning Nuclear Tests

THROUGHOUT MAY 2010, A REVIEW OF THE TREATY ON THE NON-PROLIFERATION OF NUCLEAR Weapons (NPT) is taking place at the United Nations, where its nearly 200 parties are considering the treaty's "state of health" and ways to eliminate nuclear weapons. This follows intense activity last month, in which the United States took important steps toward this goal. So it's crucial that the next important step not be missed: fully implementing the Comprehensive Nuclear-Test-Ban Treaty (CTBT). This requires U.S. ratification.

In his Prague speech a year ago, President Obama affirmed the U.S. commitment to a world free of nuclear weapons. Last month, he outlined a path for achieving this goal in the Nuclear Posture Review, sending a strong message that the role of nuclear weapons in the country's defense strategy would be reduced. This was bolstered by signature of the U.S.-Russian New Strategic Arms Reduction Treaty, in which both countries agree to lower limits on the numbers of nuclear warheads and delivery systems. And at the April Nuclear Security Summit, the United States stressed the need to address nuclear terrorism around the globe. There is now new momentum toward global nuclear disarmament, driven by an administration that takes it seriously.

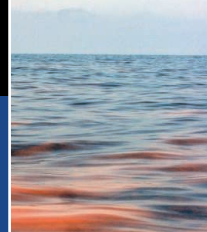
Yet the ban on nuclear tests—the next such step toward this goal—has still not been ratified by the United States. Originally proposed a half-century ago, the CTBT was eventually opened for signature in 1996, building on decades of scientific, technical, and diplomatic efforts. As of 2009, 151 nations, including all U.S. NATO allies and Australia, Japan, and South Korea, have ratified the treaty. Unfortunately, the U.S. Senate declined to approve it when it was hastily brought up for a vote in 1999. The main concerns were the treaty's value for arms control and nonproliferation, confidence in U.S. nuclear weapons in the absence of testing, and effective verification of the ban.

The Senate has good reasons to change its mind now, especially given developments since 1999. Under the CTBT, nuclear-armed states cannot advance their nuclear weapon technologies, and states seeking nuclear weapons cannot progress beyond primitive untested devices. The ban's function as a qualitative constraint is viewed by NPT parties as essential for the continued viability of the nonproliferation regime. The Stockpile Stewardship Program has ensured that U.S. nuclear weapons have been maintained in good condition over the 18 years since the U.S. testing moratorium began. But moratoriums (other nuclear-armed states have also declared them) are weak reeds, lacking the stability of a legally binding agreement. New independent assessments show that confidence in the reliability of the stockpile will be maintained so long as adequate funding and scientific talent are devoted to the task. As for verification, the United States monitors for nuclear tests with seismometers, satellites, and other technologies. Such means are better now than in 1999. The Vienna-based commission preparing for CTBT implementation is provisionally operating an international monitoring system of seismic, radionuclide, hydroacoustic, and infrasound sensors. International studies released in 2009 show that this system performs better than expected and that the ability to conduct on-site inspections is within reach. With today's verification capabilities, there is a real probability of detecting a cheating attempt at any yield.

It would be seriously destabilizing if any nuclear-armed state were to resume testing, a risk that entry into force of the CTBT, and thus its full implementation, would mitigate. The treaty cannot enter into force without the United States, and many of the nations who must approve it by terms of the agreement, but who have not yet ratified it—China, Egypt, India, Indonesia, Iran, Israel, North Korea, and Pakistan—are unlikely to act before the United States does. U.S. leadership on the CTBT is essential, and it needs to be exercised now to achieve a lasting end to nuclear tests.

—Pierce S. Corden





GENOMICS

Synthetic Genome Brings New Life to Bacterium

For 15 years, J. Craig Venter has chased a dream: to build a genome from scratch and use it to make synthetic life. Now, he and his team at the J. Craig Venter Institute (JCVI) in Rockville, Maryland, and San Diego, California, say they have realized that dream. In this week's *Science Express* (www.sciencemag.org/cgi/content/abstract/science.1190719), they describe the stepwise creation of a bacterial chromosome and the successful transfer of it into a bacterium, where it replaced the native DNA. Powered by the synthetic genome, that microbial cell began replicating and making a new set of proteins.

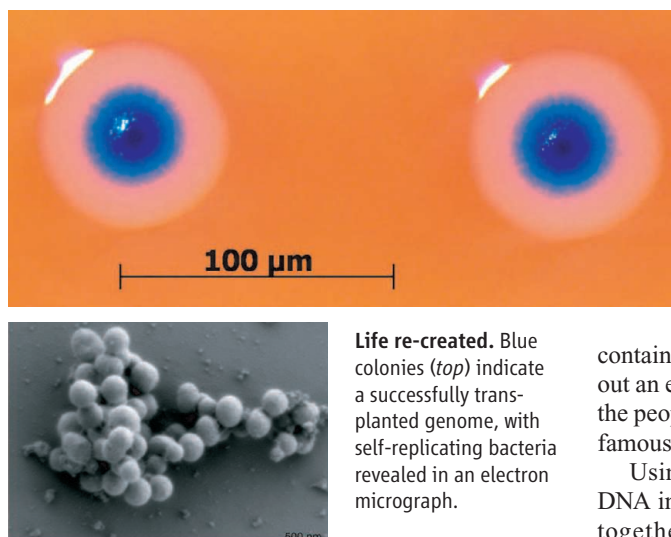
This is "a defining moment in the history of biology and biotechnology," says Mark Bedau, a philosopher at Reed College in Portland, Oregon, and editor of the scientific journal *Artificial Life*. "It represents an important technical milestone in the new field of synthetic genomics," says yeast biologist Jef Boeke of Johns Hopkins University School of Medicine in Baltimore, Maryland.

The synthetic genome created by Venter's team is almost identical to that of a natural bacterium. It was achieved at great expense, an estimated \$40 million, and effort, 20 people working for more than a decade. Despite this success, creating heavily customized genomes, such as ones that make fuels or pharmaceuticals, and getting them to "boot" up the same way in a cell is not yet a reality. "There are great challenges ahead before genetic engineers can mix, match, and fully design an organism's genome from scratch," notes Paul Keim, a molecular geneticist at Northern Arizona University in Flagstaff.

The "synthetic" bacteria unveiled this week have their origins in a project headed by Venter and JCVI colleagues Clyde Hutchison III and Hamilton Smith to determine the minimal instructions needed for microbial life and from there add genes that could turn a bac-

terium into a factory producing compounds useful for humankind. In 1995, a team led by the trio sequenced the 600,000-base chromosome of a bacterium called *Mycoplasma genitalium*, the smallest genome of a free-living organism. The microbe has about 500 genes, and researchers found they could delete 100 individual genes without ill effect (*Science*, 14 February 2003, p. 1006).

But confirming the minimal genome



Life re-created. Blue colonies (top) indicate a successfully transplanted genome, with self-replicating bacteria revealed in an electron micrograph.

suggested by those experiments required synthesizing a full bacterial chromosome and getting it to work in a recipient cell, two steps that have taken years because the technology to make and manipulate whole chromosomes did not exist. In 2007, Venter, Smith, Hutchison, and colleagues finally demonstrated that they could transplant natural chromosomes from one microbial species to another (*Science*, 3 August 2007, p. 632). By 2008, they showed that they could make an artificial chromosome that matched *M. genitalium*'s but also contained "watermark" DNA sequences that would enable them to tell the synthetic genome from the natural one (*Science*, 29 February 2008, p. 1215).

But combining those steps became

bogged down, in part because *M. genitalium* grows so slowly that one experiment can take weeks to complete. The team decided to change microbes in midstream, sequencing the 1-million-base genome of the faster-growing *M. mycoides* and beginning to build a synthetic copy of its chromosome. Last year, they showed they could extract the *M. mycoides* natural chromosome, place it into yeast, modify the bacterial genome, and then transfer it to *M. capricolum*, a close microbial relative (*Science*, 21 August 2009, p. 928; 25 September 2009, p. 1693). The next step was to show that the synthetic copy of the bacterial DNA could be handled the same way.

The researchers started building their synthetic chromosome by going DNA shopping. They bought from a company more than 1000 1080-base sequences that covered the whole *M. mycoides* genome; to facilitate their assembly in the correct order, the ends of each sequence had 80 bases that overlapped with its neighbors. So that the assembled genome would be recognizable as synthetic, four of the ordered DNA sequences contained strings of bases that, in code, spell out an e-mail address, the names of many of the people involved in the project, and a few famous quotations.

Using yeast to assemble the synthetic DNA in stages, the researchers first stitched together 10,000-base sequences, then 100,000-base sequences, and finally the complete genome. However, when they initially put the synthetic genome into *M. capricolum*, nothing happened. Like computer programmers debugging faulty software, they systematically transplanted combinations of synthetic and natural DNA, finally homing in on a single-base mistake in the synthetic genome. The error delayed the project 3 months.

After months of unsuccessfully transplanting these various genome combinations, the team's fortune changed about a month ago when the biologists found a blue colony of bacteria had rapidly grown on a lab plate over the weekend. (Blue showed the cells were using the new genome). Project leader Daniel Gibson sent Venter a text message declaring success. "I took my video camera in and filmed [the plate]," says Venter.

CREDITS (TOP TO BOTTOM): J. CRAIG VENTER INSTITUTE; T. DEERING AND M. ELLISMAN/NCMIR, UNIVERSITY OF CALIFORNIA, SAN DIEGO

Downloaded from www.sciencemag.org on May 20, 2010



Biomedicine's
big spenders

964



Evolution
of language

969

They sequenced the DNA in this colony, confirming that the bacteria had the synthetic genome, and checked that the microbes were indeed making proteins characteristic of *M. mycoides* rather than *M. capricolum*. The colony grew like a typical *M. mycoides* as well. “We clearly transformed one cell into another,” says Venter.

“That’s a pretty amazing accomplishment,” says Anthony Forster, a molecular biologist at Vanderbilt University in Nashville, Tennessee. Still, he and others emphasize that this work didn’t create a truly synthetic life form, because the genome was put into an existing cell.

At the moment, the techniques employed by Venter’s team are too difficult to appeal to any potential bioterrorists, researchers stress.

Nonetheless, “this experiment will certainly reconfigure the ethical imagination,” says Paul Rabinow, an anthropologist at the University of California, Berkeley, who studies synthetic biology. “Over the long term, the approach will be used to synthesize increasingly novel designed genomes,” says Kenneth Oye, a social scientist at the Massachusetts Institute of Technology in Cambridge. “Right now, we are shooting in the dark as to what the long-term benefits and long-term risks will be.”

As ever more “artificial” life comes into reach, regulatory agencies will need to establish the proper regulations in a timely fashion, adds Oye. “The possibility of misuse unfortunately exists,” says Eckard Wimmer of Stony Brook University in New York state, who led

a team that in 2002 created the first synthetic virus (*Science*, 9 August 2002, p. 1016).

Venter says that JCVI has applied for several patents covering the work, assigning them to his company, Synthetic Genomics, which provided much of the funding for the project. A technology watchdog group, ETC Group in Ottawa, has argued that these actions could result in a monopoly on synthesized life (*Science*, 15 June 2007, p. 1557), but others are not worried. Given the current climate for granting and upholding patents of this type, says Oye, “it is unlikely that Synthetic Genomics will become the Microsoft of synthetic biology.”

“One thing is sure,” Boeke says. “Interesting creatures will be bubbling out of the Venter Institute’s labs.” —ELIZABETH PENNISI

UNITED KINGDOM

Will Britain’s Coalition Wield the Funding Ax?

The arrival last week of Britain’s new coalition government, an unlikely union between the Conservative and Liberal Democrat parties, has brought mixed emotions for U.K. researchers. There has been a generally positive response to the choice of government ministers responsible for science. But after 13 years of a Labour administration that greatly improved the lot of scientists (*Science*, 18 May 2007, p. 965), and with the government deficit at record levels, there is grave concern that research funding will be hit. “The most important issue is to what extent cuts will fall on research and the best universities,” says astronomer Martin Rees, president of the Royal Society in London.

As the Conservative–Liberal Democrat alliance took shape, researchers were pleasantly surprised to find David Willetts as the minister for universities and science. Although his background is in the humanities, Willetts was a Conservative spokesperson for education and science during the last Parliament. In a briefing earlier this week, Willetts said: “I understand the crucial importance of blue-skies research. Scientific research can’t all be reduced to utilitarian calculations”—a very different message from that of earlier Conservative administrations. Robert Kirby-Harris, chief executive of the Institute of Physics, says he

had “very positive discussions” with Willetts during Labour’s reign. “We were impressed by him.”

Willetts’s boss at the Department for Business, Innovation and Skills, which oversees most science funding, is Liberal Democrat Vince Cable, who studied natural sciences and economics at the University of Cambridge. “They make a really strong pair of advocates” for science, says Hilary Leever, acting director of the Campaign for Science and Engineering. Cable is a member of the cabinet and Willetts will attend cabinet meetings, although he is not a member.

The most pressing concern for researchers is how their funding will fare, as the new coalition has made reducing the government deficit its top priority. The coalition has committed to cutting £6 billion from government spending this year, the broad details of which are due to be announced next week. But the specific impact on research spending is more likely to emerge



Science’s defender. David Willetts will have to fight off cuts.

from the government’s “emergency budget,” due on 22 June, or a spending review this autumn that will outline funding for the next few years. The seven U.K. research councils, which distribute grants and manage research facilities, have been asked to come up with spending plans for a variety of funding scenarios, such as flat funding, a 10% cut, a 20% cut, and so on. “A 0% cut we could live with; 20% would be a total disaster,” says U.K. physicist Ian Halliday, president of the European Science Foundation.

U.K. researchers point out that in response to the recession, some countries increased science spending as a way of boosting their economies. “Success breeds success,” says Rees. “The U.K. is strong in science, and it would be sad if anything happens to jeopardize that.” Willetts acknowledges that there are difficult times ahead. “It’s going to be tough. ... The boom has now come to an end.”

—DANIEL CLERY

CREDIT: LEON NEAL/AFP/GETTY IMAGES/NEWS.COM

ScienceNOW

From *Science's*
Online Daily News Site

Golden Years Truly Are Golden

It doesn't matter whether you're employed, whether your children still live at home, or even whether you're married. Life gets better after age 50. A new phone survey of hundreds of thousands of Americans confirms that people tend to be happier, less anxious, and less worried once they pass the half-century mark. <http://bit.ly/goldenyears>

The Scent That Makes Mice Run Scared

Even if a mouse has never seen a cat before, he'll turn tail when one is nearby. Researchers suspected that the rodents somehow sniff out their would-be assassins, but exactly what they smelled was unclear. Now scientists have isolated the compound, one of a class of urinary proteins that are secreted by cats, snakes, and a variety of other predators. <http://bit.ly/scaredmice>

Vision Cells Help Set the Body's Clock

If you like to surf the Web at night, beware: You may be affecting your body's internal clock. Humans, like other animals, rely on light cues to set their body's daily cycle of activity, or circadian rhythm. Now a new study shows that some wavelengths of light, such as those from computer screens, have an unexpectedly strong influence on these rhythms, keeping us awake, for example, when we should be sleeping. <http://bit.ly/bodyclock>



A Crack in the Mirror Neuron Hypothesis of Autism

Brain cells thought to underlie our ability to understand one another work just fine in people with autism spectrum disorders (ASD), according to the authors of a controversial new study. Other researchers had proposed that these cells, called mirror neurons, malfunction in people with ASD, disrupting their ability to understand what someone else is experiencing. If the results hold up, researchers will need another way to explain the social deficits that characterize the disorder. <http://bit.ly/mirrorneuron>

Read the full postings, comments, and more at news.sciencemag.org/sciencenow.

BIOMEDICAL RESEARCH

Former NIH Director Varmus
To Head Cancer Institute

Harold Varmus will be returning to Washington, D.C., to run the world's biggest cancer research program. President Barack Obama announced on 17 May that he plans to appoint the Nobel Prize-winning molecular biologist director of the \$5.1 billion National Cancer Institute. Varmus, now president of Memorial Sloan-Kettering Cancer Center (MSKCC) in New York City, will replace current NCI Director John Niederhuber, a Bush appointee, who expects to remain at NCI as an intramural researcher.

The choice is not a surprise: Varmus's appointment has been rumored for weeks, although in early March he told *Science* that the idea was "far-fetched." (He had announced in January that he would soon step down as MSKCC president.) But it is a surprise that Varmus, age 70, who directed the entire National Institutes of Health (NIH) from 1993 to 1999, would return to head the largest of its 27 institutes. Varmus, who was part of an informal search committee for NCI director that was having trouble finding an interested candidate, said the idea seemed "crazy" when a close friend suggested it, but he changed his mind: "Running the world's largest research

program in cancer at a time when cancer research has never been more interesting seemed like a pretty good thing to do in the next several years."

The most urgent task Varmus will face at NCI, however, will be to set priorities in an era of flat budgets. For example, some researchers have questioned the value of "big biology" projects such as The Cancer Genome Atlas, a tumor mutation sequencing project that has cost \$375 million so far. Varmus will also find on his desk an Institute of Medicine report calling for an overhaul of NCI's inefficient and underfunded cooperative groups, which have conducted many key cancer clinical trials. Varmus says he expects to review NCI's "entire portfolio."

NIH Director Francis Collins, who once worked under Varmus as head of the genome institute, praised the appointment. Collins said in a statement: "It is exhilarating and gratifying to have my good friend and colleague back at NIH. ... Harold brings unmatched expertise at all levels."

Cancer researchers seemed thrilled. Varmus is "more qualified for this position than anyone else in the universe," says can-

U.S. SCIENCE POLICY

House Blocks Bill
To Boost Research
Spending

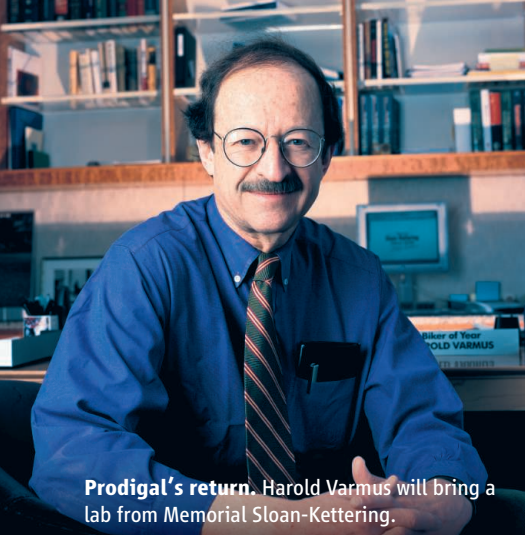
Too much sex. Not enough money. That potent combination led the U.S. House of Representatives last week to reject legislation aimed at boosting research and education spending at three federal agencies.

The 292-to-126 vote against the America COMPETES Act was fueled by concerns about the trillion-dollar federal deficit, a sentiment that could threaten the Administration's plans to double the research budgets of the three agencies over 10 years. But the lopsided defeat was also the result of a stealth attack by Republicans against a Democratic priority, using as a tool the National Science Foundation's handling of employees who trafficked in electronic pornography at work.



Can't COMPETE. Gordon (left) and Bement lament delay to House bill.

Reauthorization of the 2007 COMPETES Act is a top goal for the year of retiring Representative Bart Gordon (D-TN), chair of the House Science and Technology Committee. The version that Gordon brought to the floor last week would have kept NSF, science programs at the Department of Energy (DOE), and the National Institute of Standards and Technology on a path for a 10-year budget doubling. It also added several new programs to foster innovation and bolstered the fledgling Advanced Research Projects Agency—



Prodigal's return. Harold Varmus will bring a lab from Memorial Sloan-Kettering.

cer geneticist Bert Vogelstein of Johns Hopkins University in Baltimore, Maryland. "It's a spectacular coup on the part of the government," said cancer biologist Robert Weinberg of the Massachusetts Institute of Technology in Cambridge. Edward Benz, director of the Dana-Farber Cancer Institute in Boston, says Varmus's "impeccable credentials" as a basic scientist, his time at NIH, and his decade of directing a cancer center give him "the ideal combination of experience."

Varmus shared the 1989 Nobel Prize in physiology or medicine with Michael Bishop for using a retrovirus to understand cancer-causing genes. During a tenure at NIH that included the start of a 5-year budget doubling, he cultivated the image of a ruffled academic who biked to work. At Sloan-Kettering, he

oversaw an expansion of translational research programs and made key recruits. After endorsing Obama as a candidate in 2008 and advising the campaign, Varmus was named co-chair of the President's Council of Advisors on Science and Technology in 2009.

Despite the praise for Varmus, some insiders are worried that his appointment signals an effort to curb the independence of NCI. Under the 1971 Cancer Act, the NCI director reports directly to the president and submits a draft budget directly to the White House. The bypass budget, as it's known, is mainly symbolic but still cherished by insiders. Varmus says he has no plans to push for congressional action to change NCI's special status. But "the reality is that NCI is part of NIH, and I plan to be part of the NIH." A working group of advisers is currently examining NCI's special status, possibly to defend it. "Many people in the cancer community are concerned," says medical oncologist Richard Schilsky of the University of Chicago Medical Center in Illinois, who chairs NCI's Board of Scientific Advisors.

Varmus's return to government will mean a sharp pay cut for him from the \$2.7 million he was reportedly earning at Sloan-Kettering to an NIH salary that usually tops out at about \$300,000. The appointment does not require Senate confirmation. Varmus, who is bringing a small lab with him, expects to start at NCI on 12 July. —JOCELYN KAISER

Energy at DOE. Gordon had already pared almost \$10 billion from an earlier version of the bill to accommodate Republican opponents, and he won bipartisan support within the committee for a 5-year, \$82 billion bill.

But Gordon was caught by surprise when ranking member Representative Ralph Hall (R-TX) instead proposed a 3-year freeze on spending at those agencies. Hall's amendment included language prohibiting payment of salaries to anyone who has been disciplined "for viewing, downloading, or exchanging pornography" on a government computer or at work. Although the amendment doesn't mention NSF, freshman Representative Lynn Jenkins (R-KS) cited a 2007 investigation by the NSF inspector general of employees who had engaged in such activity, flagging it as one of many agencies where the misuse of computers had occurred. Some 121 Democrats voted for the amendment to avoid being tagged as a friend of pornography before Gordon pulled the bill.

Roughly a dozen people were found to have committed "serious infractions" involv-

ing pornographic material on their computers, says NSF Director Arden Bement, who estimates that "three or four" remain at the agency. "We nipped this in the bud," says Bement, citing "aggressive steps" by the agency to clarify its policies and impose tighter filters. "But the law is kinda murky in this area," he adds. Some of the cases "involved addiction, in which the people received counseling," he notes, and several went to arbitration. He called pornography "a pernicious problem at many agencies" that requires "clear guidance from Congress."

At presstime, Gordon was hoping to get a second shot this week at passing the bill. But given the pressure to curb spending, the U.S. research community will need to work hard to persuade Congress that an increased investment is essential for the country's economic prosperity. And as an authorization bill, COMPETES at most offers guidelines for spending panels. "I hope that it's not an omen for what may happen with appropriations," says Samuel Rankin of the Coalition for National Science Funding. —JEFFREY MERVIS

ScienceInsider

From the Science Policy Blog



A pretrial hearing before a federal judge in Tennessee was the most formal legal test yet for the use of **lie-detection technology** based on functional magnetic resonance imaging (fMRI) of brain activity. The hearing revealed new details about methods used in commercial fMRI lie-detection services; a ruling on whether to allow such evidence will establish an important precedent.

The defendant in the case, accused of defrauding Medicare and Medicaid, hired a Massachusetts-based company that provides brain-scan lie-detection services to help establish that he is telling the truth. <http://bit.ly/9H2azM>

Biologist Dylan Evans was sanctioned for **sexual harassment** by the president of University College Cork in Ireland. Evans had showed a scientific paper that revealed that bats perform fellatio to several colleagues, one of whom protested. Evans says he was just sharing information, but the college's president said his behavior was "unacceptable." <http://bit.ly/damC6R>

The U.S. Supreme Court decided Monday that **life sentences for most young criminals** amount to cruel and unusual punishment. The decision in *Graham v. Florida* extended a 2005 ruling that banned the death penalty for juveniles. The American Medical Association, American Psychological Association, and other professional organizations submitted legal briefs that questioned whether youths should be held to the same standards of culpability as adults given research suggesting that their brains are still developing. <http://bit.ly/dCR8sE>

Senators Joseph Lieberman (ID-CT) and John Kerry (D-MA) have introduced the American Power Act, which would require the country to reduce **greenhouse gas emissions** by 17% below 2005 levels by 2020. The House of Representatives passed an equivalent bill last year, but the challenge for Senate Majority Leader Harry Reid (D-NV) will be gaining the 60 votes needed to thwart a filibuster in an election year. It's unclear how much political clout the White House is prepared to exert to pass the legislation. <http://bit.ly/bTL297>

See the full postings and more at news.sciencemag.org/scienceinsider.

GULF OIL DISASTER

Five Questions on the Spill

Sizing up the weeks-long spill in the Gulf of Mexico and its impacts is proving a challenge for marine and coastal scientists. The source is beneath 1600 meters of seawater, the winds and currents spreading the oil can be capricious, and the marine life in the oil's path is spread over hundreds of square kilometers, from the sea floor to the surface. Commercial fisheries have already been affected, and fragile coastal marshlands are at risk. Just monitoring all these ecosystems is the first challenge; gauging the toll taken and sounding the all clear will come later. Here are five of the key questions that scientists will be trying to answer over the coming months and years.

—RICHARD A. KERR, ELI KINTISCH, LAURA SCHENKMAN, AND ERIK STOKSTAD

For continuing coverage of the oil spill, see <http://news.sciencemag.org/oilspill/>

What's happening ... to the oil?

The magnitude of the catastrophe will depend on the oil's fate: the amount of oil released, how the oil is transformed chemically and physically, and how far and wide it travels. To date, scientists are far from answering any of these questions.

The oft-cited 5000 barrels per day of oil spewing from the leaking well is almost certainly an underestimate. Scientists eyeballing videos of the sea-floor gusher or gauging the extent of the surface slick in satellite images see five or even 10 times as much oil coming out 3.5 weeks into the disaster. Continued analysis will improve those estimates, and ongoing efforts to stanch the flow may be informative, but as one oceanographer puts it, for now, "it is what it is."

Researchers have a better handle on how the oil is "weathering." Samples collected from the sea surface show that, as expected, the oil is tending to lose its more volatile—and more toxic—components as it evolves from a simple liquid to an emulsified "mousse" to tarballs. Although well-aged tarballs are the least damaging form of lingering oil, those starting to appear on beaches are still so sticky that plants and animals could suffer greatly. Detergent-like dispersants applied offshore accelerate both physical and biological weathering, but chemists have yet to see obvious signs that dispersants are helping. Then there are biologists' concerns that dispersants could be affecting marine life in the open gulf directly through their toxicity or indirectly by causing more of the oil to linger far below the surface where fish and bottom-dwellers are.

Researchers could soon get a better idea of what's happening to the oil and dispersants as well as where it's all going as field sampling gets in gear. Reports of large subsurface plumes of oil—perhaps enhanced by dispersants—are beginning to come in as sampling from boats and ships is extended to the subsurface. And two different autonomous underwater vehicles are scheduled to start mapping subsurface oil using optical sensors; one of them can return water samples for detailed



analysis. The as-yet-loosely-coordinated effort to characterize the evolving spill is being conducted by the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Geological Survey, while the National Science Foundation is supporting fieldwork through rapid-response grants.

CREDITS: (LEFT) CHARLIE VARLEY/SPA PRESS/NEWSOM; (RIGHT, TOP AND MIDDLE) CHRISTOPHER BERKEY; (BOTTOM) TODD WALSH © 2007 MBARI

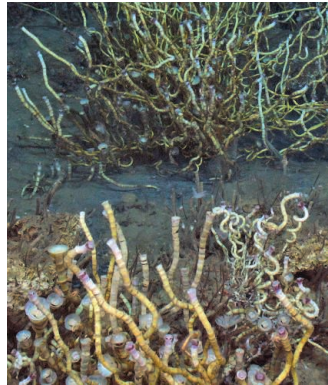
Downloaded from www.sciencemag.org on May 20, 2010

... to life on the sea floor?

Two types of communities exist on the deep sea floor of the gulf. Where hydrocarbons seep out of the sediment, clams and mussels live with symbiotic bacteria that tap sulfide or methane for energy. In the same areas, polychaete tubeworms grow up to several meters long and can live for centuries. Elsewhere, corals capture prey that floats by or detritus that sinks from above.

Expeditions have sampled sea-floor biota in the Gulf of Mexico for years with submersibles and ship-borne devices. In mid-May, a research vessel operated by the National Institute for Undersea Science and Technology (NIUST), a university consortium, began taking sediment and water samples from areas under and near the oil spill. Another group already had a passive sediment-sampling device in place nearby. When they retrieve the device, by October at the latest, they will know how much oil has settled onto the sea floor, either directly or entrained in seaweed.

Most sea-floor studies to date have been funded by NOAA and the Minerals Management Service. NOAA is supporting the NIUST mission, which detected oil below the surface. The crew returned to port late last week and will begin analyzing samples. Meanwhile, they are beginning to plan another trip for follow-up sampling.



... to coastal ecosystems?



Coastal wetlands in the Gulf of Mexico have been under siege for decades. Chronic exposure to large amounts of oil could worsen their plight, killing marsh grasses and the creatures that live in the coastal sediment.

Three weeks ago, academic researchers took sod samples from an established field site just east of the Mississippi River. Now they plan to collect oiled sods from the same site and, in a greenhouse lab, compare processes such as plant growth, photosynthesis, and soil respiration. In June or early July,

the same team plans to survey sedimentation rates at 18 sites along the wetlands west of the Mississippi, an area likely to be hit by oil. A third study would assess the effects of fresh and weathered oil on different species of marsh plants; scientists say oil that has seeped into the soil and comes in contact with roots could have greater long-term impacts on vegetation than oil slicked on the surface.

In the shallow waters of Louisiana's Breton Sound, where oil has already intruded, effects on marine life may already be visible. A team will collect live mollusks for tissue analysis, examine their shells for changes in growth rates, and look for deformities in the husks of foraminifera, an amoebalike bottom-dweller, and for large numbers of hibernating dinoflagellates in the soil.

... to marine life?

Just after the spill, researchers at the state-funded Dauphin Island Sea Lab off the Alabama coast stepped up their existing research to trawl for plankton along a 56-kilometer stretch south of the island; they will repeat the survey every 2 weeks. Another ongoing study based on rigs near the spill uses underwater, company-owned robots to monitor the squid, crustaceans, and fish that dwell 200 to 2400 meters below the surface. Besides tallying deaths, academic scientists will look for changes in the animals' daily feeding movements.

An ambitious new study is about to start thanks to a rapid-response grant from the National Science Foundation. Working from two or three university research vessels, scientists from several institutions will trace oil over the next 3 to 6 months as it moves through the food chain from single-celled algae to large fish such as tuna.

NOAA is monitoring the spill's effects on the more than 20 species of marine mammals, notably bottlenose dolphins and endangered sperm whales, and five species of endangered sea turtles that call the gulf home. Besides checking for oil in and on the bodies of six dolphins and more than 100 sea turtles collected so far, the agency is conducting aerial surveys to count the gulf's dolphins and whales, and taking biopsies of one bottle-nosed dolphin population to determine baseline levels of the animals' exposure to oil and other contaminants. Scientists will also monitor mammals acoustically with an underwater device.



... to fisheries?

On 18 May, NOAA shut down fisheries in a 118,000-square-kilometer area in the gulf. The move has threatened the lucrative shellfish industry. But the government says it is crucial to protect people from dangers of eating shellfish contaminated with polycyclic aromatic hydrocarbons, elements of oil that are carcinogenic.

Scientists are scouring the area for tainted catch—so far, with the oil still offshore, none has been found—a tricky task in itself. Current analytical methods take days. So scientists at AOAC International, a nonprofit analytical chemistry group in Gaithersburg, Maryland, are working with testing companies to try to develop faster methods for preparing and analyzing samples with mass spectroscopy.

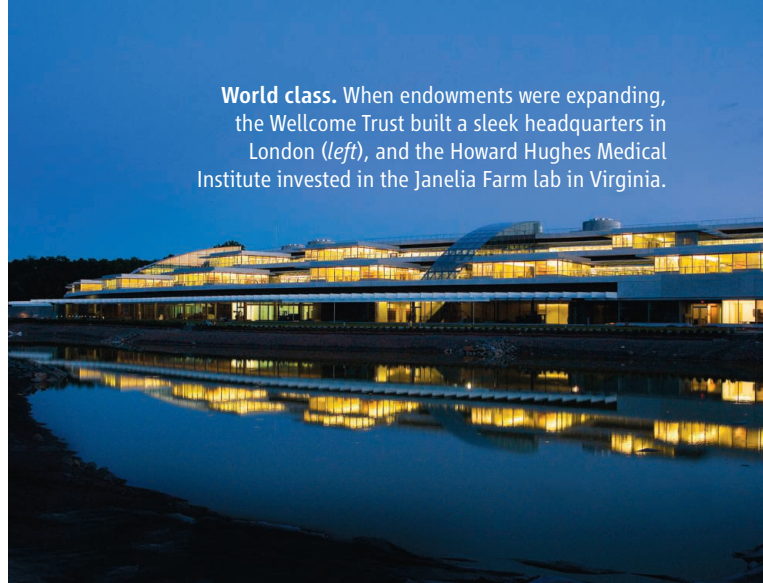
A far more difficult task will be determining when it is safe to reopen the fisheries. After previous spills, NOAA has reopened fisheries when normal background levels of oil were detected in fish or shellfish samples. But given the size of the fishery affected and its critical importance to local livelihoods, such a strict standard may be unrealistic. Former Food and Drug Administration regulator David Acheson says the agency may have to develop new standards to certify fish that contains tiny amounts of oil above trace levels. But that could take "a very long time," he says. "We don't really know what's safe."



CREDITS (TOP TO BOTTOM): DAVE MARTIN/AP PHOTO; ERIK CORDES/LOPHELIA II 2009 EXPEDITION; HANS DERYK/REUTERS/LANDOV (2)



World class. When endowments were expanding, the Wellcome Trust built a sleek headquarters in London (left), and the Howard Hughes Medical Institute invested in the Janelia Farm lab in Virginia.



Battle of the Titans

For the Howard Hughes Medical Institute and the Wellcome Trust, a rough decade on the stock market makes for straitened times

LOUDON COUNTY, VIRGINIA, AND SOMERS Town, London, sit at opposite ends of the social spectrum. Loudon County is, in terms of median household income, the wealthiest of the 3141 counties in the United States. Somers Town is one of the poorest quarters of inner London, a working-class community hemmed in between the railway lines feeding into Kings Cross station.

It is in Loudon County that the Howard Hughes Medical Institute has opened one of the largest freestanding biology laboratories in the United States, at Janelia Farm, which will eventually house about 500 people. In Somers Town, meanwhile, the Wellcome Trust is planning to help build the UK Centre for Medical Research and Innovation (UKCMRI), where 1500 scientists and support staff members will work cheek by jowl with London's people, hospitals, and universities.

The different choice of neighborhood reflects the respective personas of the two largest biomedical research philanthropies in the world. Hughes is often seen as aloof and apart, and almost entirely dedicated to excellence in basic science. It keeps a safe distance from contentious public health and policy issues.

Wellcome, in contrast, gets stuck right into almost every aspect of health research and policy. It supports the study of the history and sociology of medicine, gives grants to TV producers, seeks to influence policy, and collaborates deeply with the U.K.

Medical Research Council (MRC) and other public bodies. Yet last November, Mark Walport, chief executive of the Wellcome Trust, signaled an important convergence in approach with that of Hughes. Wellcome, he said, would end its main grants for biology projects and programs in the United Kingdom—worth about £110 million annually—and instead support selected investigators for up to 7 years.

The switch both excites and alarms British universities, where almost 3000 researchers currently receive Wellcome grants. "This is a massive change for us," says Jonathan Weber, head of medical research at Imperial College London. "We relish the opening for our senior investigators. But we're anxious about the younger ones, since we lose the project grants that were the first step on the ladder for them."

Hughes's new president, University of California, Berkeley, molecular biologist Robert Tjian, meanwhile, is trying to coax a \$500 million investment in Janelia Farm to bear fruit. The laboratory is a major departure for an organization that had always backed researchers inside existing universities or medical schools. The first 33 investigators are now bedding in at Janelia Farm, testing the bold prediction of its director, geneticist Gerald Rubin, that it can replicate the special environments of Bell Labs or the University of Cambridge Laboratory of Molecular Biology (LMB) in their heyday. Detractors see it as a gamble, however.

Financial pressures have curtailed the rapid expansion of both Hughes and Wellcome. Huge dips in asset values in 2001 and again in 2008 left both sitting on little more cash at the end of the decade than at its start (see p. 966).

At the same time, the two titans appear to be converging on a model in which excellent support for a small number of top university investigators, plus generous support for one or two intramural laboratories, consumes the bulk of their resources. Paul Nurse, the president of Rockefeller University in New York City and president-elect of the Royal Society in London, advises both philanthropies and applauds what he sees as Wellcome's shift in this direction. "Science in the U.K. has been held back by not having anything like the Hughes investigator program," he says. "Hughes is now generating one or two Nobels every year; Wellcome hasn't been doing that."

Billionaire's bequest

The Howard Hughes Medical Institute was created by the aviation billionaire back in 1953, mainly to provide a tax shelter for the Hughes Aircraft Co., whose entire stock it owned. Its research activities were modest until 8 years after Hughes's death, in 1984, when an imaginative Delaware judge, seeking to end a dispute over the estate, named eight prominent people as its trustees. They sold the corporation to General Motors the following year for a cool \$5 billion, which became the institute's initial endowment.

The structure of Hughes was novel: It was constituted as a research organization, not a foundation, and rather than offering grants or fellowships, it hired principal investigators inside the universities and medical schools as staff members. Under an agreement with the tax authorities, it was obliged to spend, on average, at least 3.5%

of its worth annually through this mechanism and an additional \$500 million over a 10-year period on related activities. Hughes trustees have chosen to continue these activities, such as grants and education, at about 1% of the endowment.

Wellcome has no such constraints but arrived at a similar spending rate—4.5% of the endowment—for a larger annual budget of £725 million (\$1.1 billion) compared with \$830 million for Hughes.

Donald Fredrickson, a former director of the U.S. National Institutes of Health (NIH), was appointed president by the new Hughes board and planned its move from Florida to a plush new headquarters in Chevy Chase, Maryland, near NIH, just north of Washington, D.C. But he left in 1987 after a scandal. (His wife was alleged to have meddled in Hughes management and to have created an unauthorized \$200,000 account.) Fredrickson was succeeded by virologist Purnell Choppin, who hired Maxwell Cowan, a University of Oxford-educated South African neuroscientist, as scientific director.

Choppin, who still has an office at Hughes, and Cowan, who died in 2002, stopped asking universities to appoint Hughes investigators, as they had done before, and instead selected them directly, with the help of topflight review panels. Cowan in particular set the tone: gentle-mannered, elitist, and almost ascetic, he believed that truly important science was only done by a talented few. “Max was enormously important and in many ways the key person in elevating the standard of quality at Hughes,” recalls former University of Chicago president Hanna Gray, the only surviving member of the original board of trustees and now its chair.

Hughes’s investigator model is a turbocharged version of the system used to promote scientists up the road at NIH, although Hughes has avoided entanglements with that agency. “NIH and Hughes were both doing their own thing,” says Choppin, “and it seemed logical to keep it that way.” With the arrival in 2000 of geneticist Thomas Cech as president, and Rubin as biomedical research vice president, Hughes gave even more autonomy to its investigators and broadened out into areas such as chemistry and computer science. “It became less formal and gave us more leeway,” recalls Bert

Vogelstein, a cancer geneticist and Hughes investigator at Johns Hopkins University in Baltimore, Maryland.

Hughes has sometimes been criticized as too cautious—simply backing the very best people, who would win NIH grants anyway. “They just sit there and skim the cream,” as one senior NIH official, who didn’t want to be identified, puts it. But for those who are selected, there are immense advantages: reliable core funding and unprecedented freedom. “It’s recognized around the world as the most effective way there is of supporting high-quality science,” says Nurse.

More than one-third of Hughes’s current investigators are members of the elite National Academy of Sciences, and 14 have won Nobel Prizes. An as-yet-to-be-published National Science Foundation-supported study by economists Pierre Azoulay and Gustavo Manso of the Massachusetts Institute of Technology and Joshua Zivin of Columbia University finds

the impression that money doesn’t matter, and that’s not a good lesson for them,” says the former Hughes scientist. Tjian says he’s been aware of this problem and has changed the rules so that each investigator can carry over \$200,000.

With four-fifths of its funding going to its 350 U.S. investigators, Hughes maintains a small headquarters staff of about 200 people. It has established an international program supporting, at different times, researchers in Eastern Europe, Latin America, and Canada. But this program is currently under review and likely to be reshaped to create closer ties to host institutions and more involvement in Asia. And it runs a \$40 million undergraduate education program, which has infused more research into science education at U.S. universities for 4000 students each year (*Science*, 16 April, p. 294).

The Hughes board’s desire to do something that NIH could never do led to its 2000 decision to buy a site at Janelia Farm for a laboratory of its own (*Science*, 8 December 2006, p. 1530). “Whereas the Wellcome Trust is a major part of scientific infrastructure in Britain, we are just 2% of the NIH budget, and maybe 5% of basic biomedical research,” says Rubin, the laboratory’s intellectual architect and now its director. “If we’re going to have an impact, we need to do things that NIH is not doing.”

The Janelia Farm project fits that bill, Rubin says, by seeking to collect a group of unusually talented investigators and place them in a sublime research environment. “Our hypothesis was that there’s something about the environment at Bell Labs and at the LMB at Cambridge that was special, that brought out the best

in people,” he says.

Small research groups are fundamental to the mix, says Rubin, adding that successful investigators at universities often end up with so many people to supervise that they can’t really do science themselves: “If you’re going to be a scientist, working with your own hands,” he says, “then we’re pretty much the only show in town.” The laboratory focuses on neuroscience, with a special emphasis on developing technologies to support neuroscientists.

Sean Eddy, a computational biologist who is working on algorithms to find homologous gene sequences, decided to move to the lab the day Rubin presented the idea to

Wellcome Trust.

A drug company founded by Henry Wellcome provided the nest egg for a £13 billion charity in his name, directed today by Mark Walport.



Howard Hughes Medical Institute.

Howard Hughes’s tax shelter evolved into a \$14 billion medical institute; its current president is Robert Tjian.



that very talented young researchers who become Hughes investigators “produce high-impact papers at a much higher rate” than those who don’t.

The downside is that they must undergo a bruising review every 5 years. “The system creates a huge amount of pressure and anxiety,” says one former Hughes investigator. At least the pain is brief: Investigators get a 45-minute presentation and 15 minutes of questions before a star-studded review panel decides whether to jettison them.

Another doubt about the model concerns the generosity of the money, which sometimes needs to be spent in a hurry, especially at year’s end. “The young people get

HHMI
HOWARD HUGHES MEDICAL INSTITUTE



1946 Howard Hughes suffers long convalescence after plane crash.

1951 Hughes appoints and funds six medical research fellows.



1953 Institute created to hold all shares in Hughes Aircraft Co., with Howard Hughes as sole trustee (pictured with actress Ava Gardner).

1930

1935

1940

1945

1950

1955

1960

1965

1970

wellcome
trust



1936 Trust established by the will of Henry Wellcome, holding all shares in the drug company.



1945–75 A collaboration between Gertrude Elion and George Hitchings pioneers rational drug design at Wellcome Foundation laboratories in New York, transforming the company and eventually winning the 1988 Prize.

1967 £2.5 million awarded for university research to prevent U.K. "brain drain" to the U.S.A.

Hughes investigators. "The whole audience was hostile, but I thought: They're building my dream," he recalls. "I just walked up to Gerry and said, 'Sign me up!'"

Rubin has also attracted problem-solvers with nonacademic backgrounds such as Eugene Myers, the computer whiz who helped Celera produce a draft human genome; Eric Betzig, an acclaimed young physicist who dropped out of Bell Labs 10 years ago to work in his father's machine shop in Michigan; and Timothy Harris, a Bell Labs veteran now heading up an applied physics group at Janelia Farm.

Not everyone agrees that this decades-old model will work, however. According to Eddy, one LMB Cambridge veteran told Rubin that if he wanted to recreate that laboratory's original environment, he should build "a hut in a swamp." And most Hughes investigators were strongly opposed to the idea of such a large, stand-alone lab—especially since the project won final approval after the 2001 market crash, when the investigators were being asked to absorb sharp cuts in their own budgets.

Rubin says the Hughes investigators are coming around slowly: "It's gone from 90:10 against to 50:50." All say that it is too early to measure the success of a project that opened its doors in October 2006. Perhaps its strongest asset is Rubin's intense yet carefully reasoned pursuit of his ideal. "Our nightmare would be to recreate the Salk," he says, referring to the prestigious but conventional Salk Institute in San Diego, California, "where it isn't clear that the whole is more than the sum of the parts."

International reach

The bustling, palatial atrium of Wellcome's new headquarters on London's Euston Road houses a bigger staff than Hughes does—about 550 people—administering the trust's slightly larger and much wider range of programs.

The trust was set up through the will of the pharmaceuticals magnate Henry Wellcome in 1936, which handed it 100% ownership of his drug company. It has been active in medical research ever since, but its transformation into a scientific powerhouse only began in 1986, when the trustees began to sell the company in three tranches, leaving themselves with an endowment worth £6.8 billion in 1995.

"The Wellcome used to act like just another research council, playing second fiddle to the MRC," recalls David Cooksey, a venture capitalist whose 2006 report to the British government has shaped national medical research policy, and who served on the Wellcome board of trustees from 1995 to 1999. "But they revisited the will, which

says that the trust should seek to improve human health, and have become much more broadly spread."

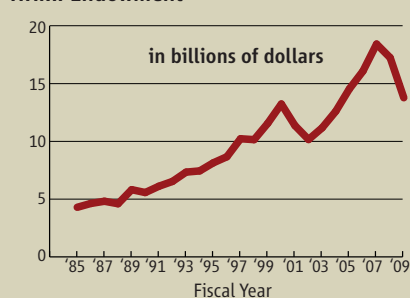
For U.K. biomedical researchers, Wellcome's mainstays are the 3- to 4-year project and program grants, which support the research activities of hundreds of university-based life scientists. The plan to replace these has come as a shock. "Once they move to the investigator awards, it is just going to be so competitive. If you've had the slightest dip in your career, you'll have no chance," says a biologist at a top U.K. university. "It's going to be very, very tough for mature postdocs to get one of these."

A 10-year strategic plan published in February unequivocally identifies the investigator awards as the first of three "focus areas" for the trust. Yet Walport denies that they mark a sharp change in direction. "This isn't a revolution," he contends. "It is an extension of our existing fellowship program, building on our history of very successful funding of people."

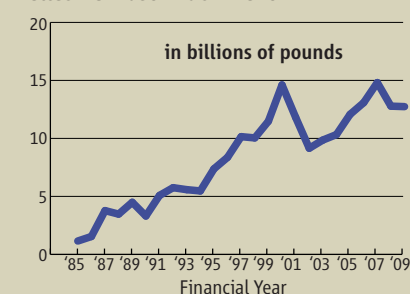
Wellcome has made the final calls for its closing grant schemes and announced on 5 May that the investigator awards will be worth between £100,000 and £425,000 a year, for up to 7 years. Walport won't say how many of them there will be: "People will need to make a case for how much money they need to answer their particular question," he says, adding that an "early investigator" category will ensure that younger scientists, at the peak of their creativity, have a fair chance.

Wellcome has a far larger international program than Hughes does, spending about £100 million a year on activities aimed at typhoid, malaria, and other tropical diseases, mainly in Southeast Asia and east Africa. These stress clinical research and capacity-building. And last September, the charity announced a £90 million joint venture with Merck to build a center for the development of affordable vaccines

HHMI Endowment



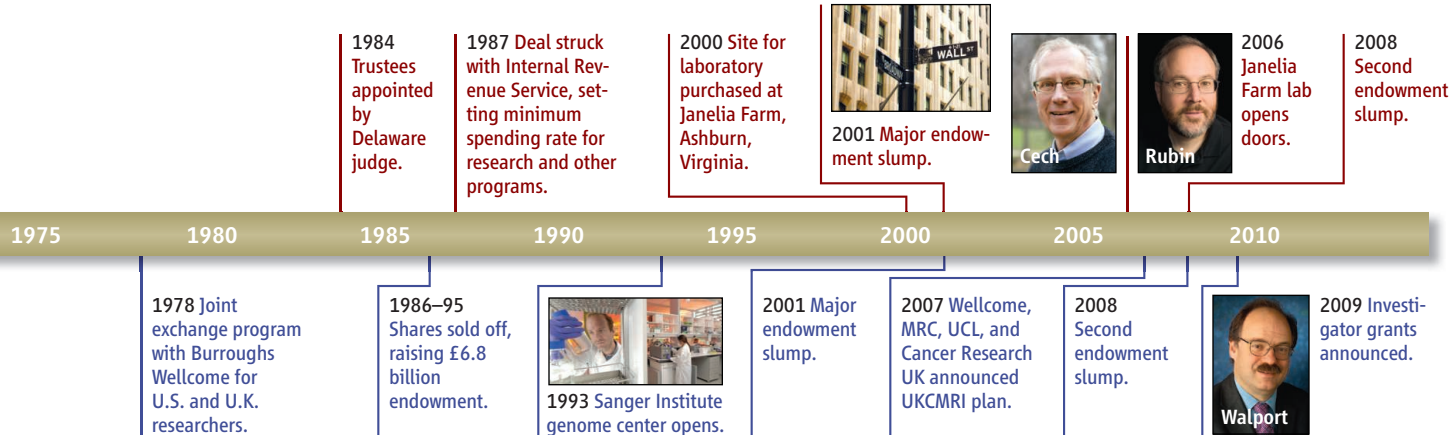
Wellcome Trust Endowment



Unsteady growth. Stock market slumps in 2001 and 2008 put a brake on the two expanding medical research empires.

CREDITS: (PHOTOS, TOP) WIKIMEDIA COMMONS; NEWSCOM; (PHOTOS, BOTTOM) WELLCOME LIBRARY; LONDON; (GRAPH SOURCES) HHMI; WELLCOME TRUST

Downloaded from www.sciencemag.org on May 20, 2010



in India. “The trust has had a long-term interest in tropical medicine, right from when Henry Wellcome had laboratories in Sudan,” explains James Whitworth, its head of international activities.

Wellcome is also more heavily involved in public outreach, spending about £40 million a year on activities ranging from the museum at headquarters—which attracted more than 300,000 visitors last year—to a regular, highly detailed survey of public attitudes toward science and medicine. In partnership with the government, it also runs a National Science Learning Centre at the University of York to do residential courses for schoolteachers: 6500 of them last year. “Our engagement program makes us different from other funders,” says Clare Matterson, the former management consultant who runs it. “I think we lead the way in this.”

Back in 1993, the trust established a major laboratory of its own, the Sanger Institute near Cambridge, to undertake the British arm of the Human Genome Project. Afterward, Sanger morphed into a large, multidisciplinary center whose 40 principal investigators do systems biology and genomics projects that take advantage of its sequencing and bioinformatics capacity.

Unlike Janelia Farm, Sanger has close ties with a nearby university—the University of Cambridge, where several of its investigators hold faculty positions—and many of its projects are collaborations with outside agencies and institutions. It gets £100 million a year from Wellcome and another £25 million from other funders. “Almost everything we do is collaborative,” says Michael Stratton, Sanger’s director, whose own work is on the Cancer Genome Project. “It tends to be the sort of work that needs to be done on a scale that can’t be achieved at other institutions.”

Wellcome’s next major capital project is the UKCMRI, a £625 million collaboration with MRC, Cancer Research UK,

and University College London, that will house up to 1500 scientists and support staff members. The laboratory won’t have a hospital, but it will be focused on work that can be applied to human health: “We’ll be trying to make sure that the status and reward systems are as tuned towards innovation as towards discovery,” says John Cooper, the project’s interim chief executive, now running a small project team from Wellcome headquarters.

UKCMRI is illustrative of Wellcome’s close ties with the U.K. government and of the risks and benefits that these carry. The government money isn’t guaranteed, for example, given the perilous state of Britain’s public finances.

A wobbly decade

Both Hughes and Wellcome have regained their financial footing after being roundly battered by the 2001 crash and then again by the one in 2008. The second blow was described in Wellcome’s 2009 annual report as “the worst decade for equity markets in over 300 years.” Hughes’s chief investment officer, Landis Zimmerman, says that the worst thing about the 2008 collapse was that it hit all investment sectors, “so that all that diversification doesn’t protect us.” The financial performance of both institutions has been drastically less impressive since 2000.

Apart from one another, the two organizations have no rival outside government of equal size or with as much power to influence biomedical science. (The Bill and Melinda Gates Foundation, which spends more overall, devotes only a small fraction to scientific research.) Wellcome is “an excellent organization and its impact is huge,” says Leszek Borysiewicz, chief executive of MRC. Its existence allows Britain to spend almost twice as much on basic biomedical research as France or Germany. Hughes has set world-beating standards that other funders have struggled to match.

“I think this is the way all research should be funded,” says Vogelstein. “It encourages the best researchers just to do the best research. You are obliged to do something really phenomenal, not just incremental.”

Comparing the two organizations by measuring their relative scientific outputs is virtually impossible. Both eschew the current fashion for quantitative assessment, for example through citation analysis. David Pendlebury, a citation analyst for Thomson Reuters and one of the world’s leading authorities on citation statistics, says that any statistical comparison will be “apples against oranges” partly because Wellcome investigators don’t identify themselves as such when listing their affiliation. “What matters in science is what has been discovered, and who’s been trained,” as Walport puts it, adding that narrative tends to describe these things better than statistics are able to.

In the end, Wellcome is larger than Hughes because it inherited more money. Hughes does more Nobel-league science, as it can cherry-pick talent from the world’s only scientific superpower. Wellcome does more within its social orbit because it has chosen to do so, and because Britain is smaller. It also does more for the developing world, because of its imperial inheritance, and because Hughes’s agreement with the tax authorities constricts its main research program to spending within the United States.

As pursuers of discovery by serendipity, it is appropriate that today’s Hughes and Wellcome owe their very existences to serendipitous events. If either one had failed to liquidate its stock bequest at the right time, in the mid-1980s, biomedical research would be much the poorer. Perhaps the greed-is-good decade, so caricatured by novelist Tom Wolfe, had its good points, after all.

—COLIN MACILWAIN

Colin Macilwain is a writer in Edinburgh, U.K.



Listen up! Both primates and birds have something to say about human language origins.

EVOLUTION OF LANGUAGE

Animal Communication Helps Reveal Roots of Language

An interdisciplinary gathering marks a turning point for a field historically richer in talk than data, but which now is increasingly embracing studies of animals

UTRECHT, NETHERLANDS—Animals communicate with each other constantly: Birds sing, monkeys chatter, and apes pant-hoot. But what they say is usually pretty simple: They want to mate, send an alert about food or predators, or express their dominance in the group. Only humans appear to have true language, the ability to use abstract symbols—usually words—and combine them in a seemingly infinite variety of meanings about the past, present, and future.

Researchers have pondered the origins of language for at least 200 years, and for much of that time, their conjectures were little more than talk. Unlike many other human behaviors, such as art and toolmaking, language leaves no traces in the archaeological record. And many researchers have been doubtful about how much animal communication could reveal about the unique features of human communication. That began to change in the 1990s, when linguists, evolutionary biologists, psychologists, primatologists, and other scientists teamed up to test new hypotheses about how language arose (*Science*, 27 February 2004, p. 1316). Since 1996, this interdisciplinary crowd has gathered every 2 years at Evolang, a meeting devoted to deciphering the evolutionary origins of language.

Although some say the early Evolang gatherings suffered from too many hypotheses and

too little testing, many think the meeting* here last month marks a turning point for the field. Participants flocked to hear a barrage of new data from animal and human studies. “The field has matured, and there is a trend towards more empirical work,” says evolutionary biologist W. Tecumseh Fitch of the University of Vienna in Austria.

One reason is that fewer scientists now follow the early views of linguist Noam Chomsky that language emerged *de novo* in humans, with little or no ape precursors. Indeed, Chomsky himself no longer holds strictly to that view, as evidenced by a seminal 2002 paper in *Science* he co-authored with Fitch and Harvard University psychologist Marc Hauser (*Science*, 22 November 2002, p. 1569), urging research into both the aspects of human language unique to humans and the aspects shared with other animals. “The more we study animals, the more we realize that they have abilities similar to ours,” says Natalie Uomini, an archaeologist at the University of Liverpool in the United Kingdom.

The new empiricism may help resolve one of the field’s liveliest debates: whether

the first human language consisted of gestures, similar to today’s sign languages, or articulated speech. And here in Utrecht, a new and unlikely seeming animal model for human language got star billing: songbirds. Their ability to learn and imitate their parents’ melodious tunes has many parallels with the ability of human children to learn spoken language, researchers say.

Hand, mouth, or both?

Pity poor Viki the chimpanzee. During the 1950s, two psychologists raised Viki in their own home like a human child and tried to teach her to speak. Viki managed a rough approximation of only four words: mama, papa, cup, and (maybe) up. The following decade, researchers had much better luck with a chimp named Washoe when they tried to teach him American Sign Language. But few scientists think Washoe’s impressive efforts represent true language (*Science*, 2 April, p. 38).

Such evidence that apes are poor at vocalizing, but fairly good at gesturing, has bolstered the so-called gestural theory for language origins. According to this model, the first human language consisted of signing, and articulate speech came later. In recent years, the gestural theory has gained the upper hand in many scientific journals and meetings. “Apes are much better at controlling their hands” than at vocalizing, says Fitch. “Their gestures are more intentional and more under control.”

Many researchers have assumed that most primate vocalizations are innate or instinctual rather than learned, and so are uninformative about the origins of human language. For example, the vervet monkey

Online

sciencemag.org

Podcast interview with author Michael Balter.

*8th International Conference on the Evolution of Language, Utrecht, Netherlands, 14–17 April 2010.

gives out specific, stereotypical alarm calls corresponding to predators such as leopards, snakes, and eagles. These calls, which are innate, are a stark contrast to the way humans combine words in novel ways.

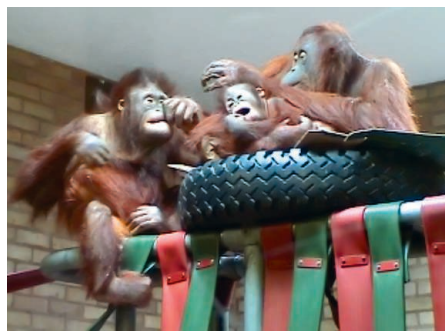
But psychologist Katie Slocombe of the University of York in the U.K. argues that the data don't support generalizing about all primate calls based on a few examples. In a poster, she and other European colleagues critiqued more than 550 studies of primate communication and found that few studies examined ape vocalization. "Absence of evidence may not reflect absence of [vocal] ability," Slocombe's team concluded.

In other posters and talks, Slocombe and others documented that chimps in the wild do vary their vocalizations in response to circumstances, a step toward language. Slocombe and psychologist Klaus Zuberbühler of the University of St. Andrews in the U.K. showed that chimps modify screams they emit when under attack depending on the severity of the aggression and their social status compared with nearby chimps. Also, wild chimps emit so-called rough grunts—vocalizations associated with the finding of food—more often when chimp allies are present and the food is of high quality.

Such findings got dramatic support from a talk on a more distantly related species, the Campbell's monkeys of the Côte d'Ivoire. Primatologist Alban Lemasson of the University of Rennes 1 in France, Zuberbühler, and their colleagues found that the males of these forest-dwelling monkeys have six different types of calls, which the researchers refer to as Boom, Krak, Hok, Hok-oo, Krak-oo, and Wak-oo. Yet these sounds are rarely used in isolation. Rather, they are combined in vocal sequences averaging 25 successive calls depending on whether the monkeys were encountering predators such as eagles or leopards, falling trees, the presence of neighboring

groups, and so forth. Moreover, the animals carried on complex "conversations" in which the call sequences were constantly being modified or altered (see *ScienceNOW*, <http://news.sciencemag.org/sciencenow/2009/12/04-02.html>).

This complexity is "significantly beyond" what researchers have assumed for nonhuman primates, Lemasson told the meeting, and is "at odds with the gestural origins of language theory." Uomini calls these studies "brilliant



Do I make myself clear? Orangutan gestures are intentional and meaningful.

work" and says the call combinations could be considered a form of "proto-language" and "proto-speech." And Erica Cartmill, a psychologist at the University of Chicago in Illinois, agrees that the Campbell's monkeys do seem to have "the ability to combine calls in different ways." But she cautions that the calls fall far short of the kind of syntactical structures typical of human languages, which have specific rules for how words can be put together into sentences.

As the vocalization camp made gains, the gesturalists had advances of their own to put forth. Numerous recent studies have underscored the importance of gestures in both human and ape communication. In most humans, brain regions specialized for language, such as Broca's area, are located in the

left hemisphere, which in right-handers also controls the movements of the right side of the body (see *Science's* Origins blog, <http://tinyurl.com/n8wroy>). Researchers are debating whether nonhuman apes also show asymmetries in homologous brain areas, and if these are the precursors of the lateralized language centers of the human brain.

Recent work by cognitive scientists Jacques Vauclair and Adrien Meguerditchian of the University of Provence in Aix-en-Provence concludes that such brain asymmetries in apes might indeed be linked to gesturing. The researchers found that baboons have a strong right-hand preference during communicative gestures such as begging for food but little hand preference during noncommunicative gestures such as wiping their faces. Captive chimpanzees show similar preferences, according to work reported by the team in *Cortex* this year.

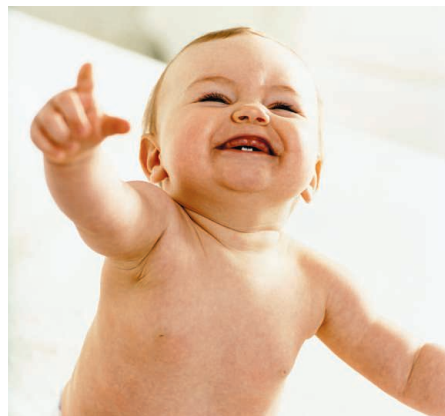
And Vauclair has recently extended such studies to human children. Infants and toddlers tend to use their right hand for pointing—a communicative gesture that appears at about 11 months of age and closely accompanies early spoken language—even if they are ambidextrous or left-handed in other situations, Vauclair's group reported in *Developmental Science* last year. This suggests that human gesture and speech are linked and that both are at least partly localized in the brain's language areas, they concluded.

In Utrecht, Vauclair's Provence colleague Hélène Cochet presented further studies along these lines. She observed the pointing behavior of 48 toddlers in French day care centers. Earlier research has established two types of pointing behavior in young children: imperative pointing, which is used to ask for something the child wants; and declarative pointing, which is used to share interest or information. Researchers consider declarative pointing to reflect more complex cognitive processes, such as understanding that other people are independent agents with their own thoughts. On the other hand, most gesturing by nonhuman apes is only imperative, such as begging for food.

Cochet found that declarative pointing was more often accompanied by spoken utterances than was imperative pointing. And although children used their right hands more often for both imperative and declarative pointing than for noncommunicative gestures such as reaching for an object, the right-handed trend was even stronger when children were declaratively pointing to provide information to an adult. "Our results suggest that such cooperative gestures may have played an important role in the evolution of language,"



Getting the point. Both apes and humans may use language-related centers in the left brain when pointing with their right hands.



CREDITS (TOP TO BOTTOM): COURTESY OF ERICA CARTMILL; ISTOCKPHOTOS.COM; GETTY IMAGES

Downloaded from www.sciencemag.org on May 20, 2010

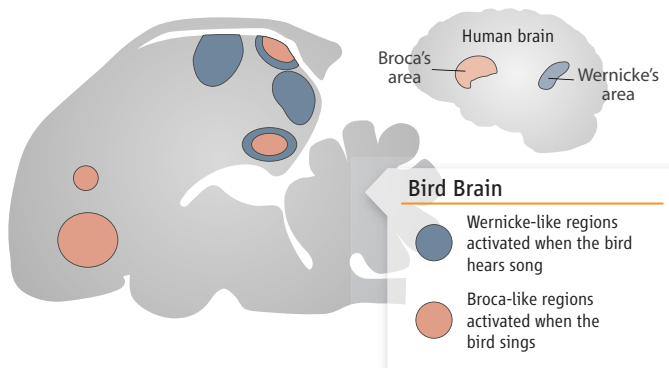
Cochet told the meeting.

To explore whether ape gestures have specific meanings, Cartmill videotaped 28 orangutans at three European zoos, accumulating more than 100 hours of recordings. She identified 37 gesture types that could be reliably assigned to one of six different meanings, such as “play with me,” “share your food,” and “go away.” Thus the apes are conveying meaning to each other with their gestures, Cartmill concluded. “Intentional, meaningful, and socially sensitive communication emerged long before” the kind of symbolic communication typical of human language, she says.

But was that early human communication primarily gestural or vocal? Each camp continues to make its case, but some researchers at the meeting urged that the field acknowledge the importance of both. “Both modalities provide potential [primate] precursors for different elements of language,” says Slocombe, “but neither of them alone can provide the complete picture.” Thus primate vocalizations are discrete signals that can be combined in sequences—as in the Campbell’s monkey—although primate gestures have the advantage of being flexible and highly intentional, Slocombe says. In his talk, Meguerditchian proposed that as early human language evolved, gestures

Vocal learner. Zebra finches learn to sing much like babies learn to talk.

CONVERGENT EVOLUTION OF VOCAL LEARNING



Singing centers. Birds may help reveal how humans learn to speak, because both species have areas of the brain specialized for vocal learning.

might initially have been more effective for “talking,” although vocalizations might have been better suited for listening. Primate gestures appear more localized to brain areas homologous to Broca’s area—implicated in speech production—whereas primate vocalizations have been more closely linked to brain areas homologous to Wernicke’s area, which is involved in the understanding and perception of speech, he pointed out.

“Gesture ... might have helped get speech off the ground over evolutionary time,” suggests psychologist Susan Goldin-Meadow of the University of Chicago. “The gesture-speech relationship we see today, where [they] work synergistically to form an integrated system, might have been there from the start.”

Birds move to center perch

Language evolution researchers have concentrated on apes and other primates because they are our closest relatives. But those animals can’t match a key feature of human language: vocal learning, the amazing ability of young children to imitate the sounds of adults. Vocal learning does turn up in a handful of other species, including whales and possibly bats, but the masters of this talent are songbirds, parrots, and hummingbirds (*Science*, 31 January 2003, p. 646).

In his talk leading off a songbird workshop, biologist Johan Bolhuis of Utrecht University listed the numerous parallels between the way songbirds learn to sing and the way human infants learn to speak. Both must be exposed to adult “tutors”; juveniles of both species have a sensitive period for vocal learning; and both young birds and human infants “babble” (called “subsong” in birds) while learning to vocalize.

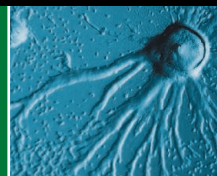
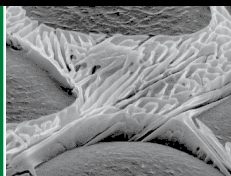
Over the past few years, Bolhuis and other researchers have traced vocal learning and song production to bird

brain areas that appear analogous to human language areas such as Wernicke’s and Broca’s areas (see diagram). These similarities are not likely to be the result of shared evolutionary history, because the lineages leading to birds and humans diverged roughly 300 million years ago. But they may prove instructive all the same, Fitch says. “To do vocal learning, you need to ... hear something and then pipe it over from the [brain’s] auditory cortex to the motor cortex,” which controls speech production, Fitch argues. “There are probably not that many different ways of getting those connections.”

Thus, Fitch says, the bird model might be able to tell us “how to build a brain that can do vocal learning.” Researchers are beginning to find some of the molecular details of how that happens. At the Evolang meeting, Kazuo Okanoya, a biolinguist at the RIKEN Brain Science Institute in Wako City, Japan, reported that genes coding for molecules called cadherins—involved in nerve cell connections in humans and other mammals—are expressed at high levels when Bengalese finches listen to adult songs and down-regulated when they start to sing themselves.

Evidence for parallels between bird song and human language continues to accumulate. Some researchers have argued that only humans are able to distinguish words that closely resemble each other. But Verena Ohms of the Institute of Biology Leiden in the Netherlands taught zebra finches to distinguish two very similar-sounding Dutch words, pecking a button after hearing the correct word of either *wit* (white) or *wet* (law), even when the words were spoken by a variety of human voices, both male and female.

Fitch says that these parallels suggest that language evolution researchers can learn a lot about human speech by studying our distantly related feathered friends. He points to recent work by animal behaviorist Constance Scharff of the Free University of Berlin and her co-workers, showing that *FOXP2*, a gene implicated in human speech, also plays an important role in bird-song learning. Fitch says that such molecules might have been recruited by natural selection to perform similar functions even in species that went their evolutionary ways long ago. Thus, despite their distance from humans, birds are now perched firmly on the Evolang agenda. Indeed, the next meeting, in Kyoto, Japan, in 2012, will be organized by bird-brain expert Okanoya and his colleagues. —MICHAEL BALTER



LETTERS

edited by Jennifer Sills

Cretaceous Extinctions: Multiple Causes

IN THE REVIEW "THE CHICXULUB ASTEROID IMPACT AND MASS EXTINCTION AT THE CRETACEOUS-Paleogene boundary" (P. Schulte *et al.*, 5 March, p. 1214), the terminal Cretaceous extinctions were confidently attributed to a single event, the environmental consequences of the impact of an extraterrestrial body. The list of 41 authors, although suggesting a consensus, conspicuously lacked the names of researchers in the fields of terrestrial vertebrates, including dinosaurs, as well as freshwater vertebrates and invertebrates. Although we the undersigned differ over the specifics, we have little doubt that an impact played some role in these extinctions. Nevertheless, the simplistic extinction scenario presented in the Review has not stood up to

the countless studies of how vertebrates and other terrestrial and marine organisms fared at the end of the Cretaceous (1–4).

Patterns of extinction and survival were varied, pointing to multiple causes at this time—including impact, marine regression, volcanic activity, and changes in global and regional climatic patterns (5). It is telling that in all other instances of mass extinction in the past 600 million years, no signature of an extraterrestrial impact has ever been reliably detected, despite extensive searches. Moreover, there are many other known instances of large impacts in the geologic record, with no associated extinctions (6). The general

Deccan plateau basalts. Lava from Deccan volcanism formed distinct layering.

importance of impacts to extinction is called into question, as well as the importance of the Cretaceous-Paleogene impact as a single cause (7). By contrast, all of the five widely accepted mass extinctions occur during or shortly after times of global marine regression (8) and at least three occur during intervals of massive volcanism (9).

J. DAVID ARCHIBALD,^{1*} W. A. CLEMENS,² KEVIN PADIAN,² TIMOTHY ROWE,³
NORMAN MACLEOD,⁴ PAUL M. BARRETT,⁴ ANDREW GALE,⁵ PAT HOLROYD,⁶

HANS-DIETER SUES,⁷ NAN CRYSTAL ARENS,⁸ JOHN R. HORNER,⁹ GREGORY P. WILSON,¹⁰

MARK B. GOODWIN,⁶ CHRISTOPHER A. BROCHU,¹¹ DONALD L. LOFGREN,¹² STUART H. HURLBERT,¹

JOSEPH H. HARTMAN,¹³ DAVID A. EBERTH,¹⁴ PAUL B. WIGNALL,¹⁵ PHILIP J. CURRIE,¹⁶ ANNE WEIL,¹⁷

GUNTUPALLI V. R. PRASAD,¹⁸ LOWELL DINGUS,¹⁹ VINCENT COURTILOTT,²⁰ ANGELA MILNER,⁴

ANDREW MILNER,⁴ SUNIL BAJPAI,²¹ DAVID J. WARD,²² ASHOK SAHNI²³

¹Department of Biology, San Diego State University, San Diego, CA 92182–4614, USA. ²Department of Integrative Biology and Museum of Paleontology, University of California, Berkeley, CA 94720–3140, USA. ³Jackson School of Geosciences, University of Texas, Austin, TX 78712, USA. ⁴Department of Palaeontology, The Natural History Museum, London SW7 5BD, UK. ⁵Department of Earth and Environmental Science, University of Portsmouth, Portsmouth, Hants PO1 3QL, UK. ⁶Museum of Paleontology, University of California, Berkeley, CA 94720–4781, USA. ⁷Department of Paleobiology, National Museum of Natural History, Washington, DC 20013–7012, USA. ⁸Department of Geoscience, Hobart and William Smith Colleges, Geneva, NY 14456, USA. ⁹Museum of the Rockies, Montana State University, Bozeman, MT 59717, USA. ¹⁰Department of Biology, University of Washington, Seattle, WA 98195–1800, USA. ¹¹Department of Geoscience, University of Iowa, Iowa City, IA 52242, USA. ¹²Raymond M. Alf Museum of Paleontology, Claremont, CA 91711, USA. ¹³Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND 58202–8358, USA. ¹⁴Royal Tyrrell Museum, Drumheller, AB T0J 0Y0, Canada. ¹⁵School of Earth and Environment, University of Leeds, Leeds LS2 9JT, UK. ¹⁶Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada. ¹⁷Department of Anatomy and Cell Biology, Oklahoma State University Center for Health Sciences, Tulsa, OK 74107, USA. ¹⁸Department of Geology, University of Delhi, Delhi 110 007, India. ¹⁹InfoQuest Foundation, 160 Cabrini Boulevard, No. 48, New York, NY 10033, USA. ²⁰Institut de

Physique du Globe de Paris, 75251 Paris, France. ²¹Department of Earth Sciences, Indian Institute of Technology, Roorkee 247667 (Uttarakhand), India. ²²Department of Earth and Environmental Sciences, Medway Campus, University of Greenwich, Chatham, Kent ME4 4AW, UK. ²³Centre of Advanced Study in Geology, Lucknow University, Lucknow 226001, India.

*To whom correspondence should be addressed. E-mail: darchibald@sunstroke.sdsu.edu

References

- G. P. Wilson, *J. Mam. Evol.* **12**, 53 (2005).
- J. D. Archibald, D. E. Fastovsky, in *The Dinosauria*, D. B. Weishampel *et al.*, Eds. (Univ. of California Press, Berkeley, CA, 2004), pp. 672–684.
- N. MacLeod *et al.*, *J. Geol. Soc. London* **154**, 265 (1997).
- P. M. Barrett *et al.*, *Proc. R. Soc. London Ser. B* **276**, 2667 (2009).
- N. MacLeod, in *Evolution on Planet Earth*, L. Rothschild, A. Lister, Eds. (Academic Press, London, 2003), pp. 253–277.
- C. W. Poag *et al.*, in *Deep-Sea Research, Part II*, R. Gersonde *et al.*, Eds. (Pergamon, New York, 2002), pp. 1081–1102.
- N. C. Arens, I. D. West, *Paleobiology* **34**, 456 (2008).
- S. E. Peters, *Nature* **454**, 626 (2008).
- P. B. Wignall, *Earth Sci. Rev.* **53**, 1 (2001).

Cretaceous Extinctions: The Volcanic Hypothesis

IN THEIR REVIEW "THE CHICXULUB ASTEROID impact and mass extinction at the Cretaceous-Paleogene boundary" (5 March, p. 1214), P. Schulte *et al.* conclude that "the Chicxulub impact triggered the mass extinction." However, the Review does not give sufficient and accurate consideration to the volcanic hypothesis. The authors claim that for Chicxulub, "the extremely rapid injection rate of dust and climate-forcing gases would have magnified the environmental consequences compared with more-prolonged volcanic eruptions." As evidence, they cite our paper (1), saying, "the injection of ~100 to 500 Gt of sulfur into the atmosphere within minutes after the Chicxulub impact contrasts with volcanic injection rates of 0.05 to 0.5 Gt of sulfur per year during the ~1-million-year-long main phase of Deccan flood basalt volcanism." This contains a substantial error and a fundamental misrepresentation of our paper. Half a Gt per year of sulfur for 1 million years amounts to 500,000 Gt of sulfur, which in any

CORRECTIONS AND CLARIFICATIONS

Reports: “Cryogenian glaciation and the onset of carbon-isotope decoupling” by N. L. Swanson-Hysell *et al.* (30 April, p. 608). A typographical error in the Fig. 2 caption was introduced to the print edition during page proofs. The correct final sentence of the Fig. 2 caption is, “An increase in k_w and in the relative burial of C as organic matter can result in a decrease in CO_2 , as shown for the Mesoproterozoic–Tonian–Cryogenian, without changes in volcanic CO_2 input.” The caption is correct in the HTML version online.

Cover caption: (23 April, p. 397). The cover image showed children studying chemistry, not nuclear physics.

Reports: “Protein kinase C- θ mediates negative feedback on regulatory T cell function” by A. Zanin-Zhorov *et al.* (16 April, p. 372). In the first sentence of the second paragraph on p. 372, T_{eff} should have been defined as $\text{CD4}^+ \text{CD25}^-$.

Reports: “Arsenic trioxide controls the fate of the PML-RAR α oncoprotein by directly binding PML” by X.-W. Zhang *et al.* (9 April, p. 240). In the legend for Fig. 3C, the local structure models were described incorrectly; Zn-PML-R is blue and As-PML-R is orange.

Policy Forum: “China’s road to sustainability” by J. Liu (2 April, p. 50). In the first sentence, China refers to the People’s Republic of China. The word “caused” was missing from the sentence “The ‘Great Leap Forward’ movement (1958–1961) caused the loss of at least 10% of China’s forests to fuel backyard furnaces for steel production.”

News Focus: “Immunology uncaged” by M. Leslie (26 March, p. 1573). The article incorrectly stated that the Center for Human Immunology, Autoimmunity, and Inflammation was part of the National Heart, Lung, and Blood Institute. It is a separate initiative sponsored by several NIH institutes. The article should also have emphasized that the center is conducting immunological research and is not a technical service facility.

News Focus: “Treatment as prevention” by J. Cohen (5 March, p. 1196). The title on the graphic mistakenly indicated that “incidence” dropped. The researchers did not measure “incidence” but did find a drop in the number of HIV infections detected.

Reports: “Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux” by Q. Wang *et al.* (19 February, p. 1004). In Fig. 2B, the inhibitor used to mimic the *cobB* mutation was NAM (nicotinamide) not NAD $^+$ (nicotinamide adenine dinucleotide).

climate model would lead to a “snowball” Earth! In (1), we estimate the total amount of SO_2 released by the traps at about 10,000 Gt, less by a factor of 50 than that in Schulte *et al.*’s Review. The Deccan erupted in a small number of short, huge pulses, reducing actual injection duration to far less than 1 million years. We and others (2, 3) have argued that two main phases likely lasted a few thousand to tens of thousands of years, during which individual flows would have reached volumes of 10,000 km^3 and released up to 100 Gt of sulfur in one or a few decades (based on analysis of paleomagnetic secular variation). Schulte *et al.* use our study to show that volcanism did not lead to the extinction, yet we showed that injection of SO_2 by a single volcanic pulse could have had a climatic impact similar to Chicxulub. We estimated (1) that the largest Deccan pulses emitted up to 100 Gt of sulfur at 0.5 Gt per year for a few decades to tens of decades, implying a radiative effect slightly lower but lasting substantially longer than in the impact case. Whereas impact was a single event, some 30 volcanic pulses emitted total amounts of SO_2 not very different from Chicxulub. Their sequence would have generated a runaway effect not allowed by a single impact or volcanic pulse. Evidence of an association between extinctions and continental flood basalts (CFBs) arising from eruption has been proposed since at least 1986 (4–7). Subsequent work has shown that all extinction or oceanic anoxia events in the past 300 million years are associated with a

large accumulation of igneous material of the same age within uncertainties (8). No conclusive impact has been demonstrated at any mass extinction boundary other than the Cretaceous-Paleogene. The case of the largest CFB (Siberian traps) and mass extinction (Permo-Triassic ~250 million years ago) is now generally accepted. Without challenging the existence or age of the Chicxulub impact, we believe that it is increasingly arguable that it could not by itself have caused a mass extinction, but that because it took place demonstrably during Deccan eruptions (9, 10), it contributed significantly to the mass extinction, as yet another giant lava flow could have, in an already very weakened environment.

VINCENT COURTILOTT* AND FRÉDÉRIC FLUTEAU

Institut de Physique du Globe, Paris, France.

*To whom correspondence should be addressed. E-mail: courtill@ipggp.fr

References

1. A.-L. Chenet *et al.*, *J. Geophys. Res.* **114**, B06103, 10.1029/2008JB005644 (2009).
2. S. Self, T. Thordarson, M. Widdowson, A. Jay, *Earth Planet. Sci. Lett.* **248**, 518 (2006).
3. G. Keller, T. Adatte, S. Gardin, A. Bartolini, S. Bajpai, *Earth Planet. Sci. Lett.* **268**, 293 (2008).
4. V. Courtillot *et al.*, *Earth Planet. Sci. Lett.* **80**, 361 (1986).
5. M. R. Rampino, R. B. Stothers, *Science* **241**, 663 (1988).
6. V. Courtillot, *Isr. J. Earth Sci.* **43**, 255 (1994).
7. V. Courtillot, *Evolutionary Catastrophes: The Science of Mass Extinctions* (Cambridge Univ. Press, Cambridge, 1999).
8. V. Courtillot, P. Renne, *C. R. Acad. Sci.* **335**, 113 (2003).
9. N. Bhandari, P. N. Shukla, Z. G. Ghevariya, S. M. Sundaram, *Geophys. Res. Lett.* **22**, 433 (1995).
10. V. Courtillot *et al.*, *Earth Planet. Sci. Lett.* **182**, 137 (2000).

Cretaceous Extinctions: Evidence Overlooked

IN THEIR REVIEW “THE CHICXULUB ASTEROID impact and mass extinction at the Cretaceous-Paleogene boundary” (5 March, p. 1214), P. Schulte *et al.* analyzed the 30-year-old controversy over the cause of the end-Cretaceous mass extinction and concluded that the original theory of 1980 was right: A large asteroid impact on Yucatan was the sole cause for this catastrophe. To arrive at this conclusion, the authors used a selective review of data and interpretations by proponents of this viewpoint. They ignored the vast body of evidence inconsistent with their conclusion—evidence accumulated by scientists across disciplines (paleontology, stratigraphy, sedimentology, geochemistry, geophysics, and volcanology) that documents a complex long-term scenario involving a combination of impacts, volcanism, and climate change. Here, we point out some of the key evidence that Schulte *et al.* overlooked.

The underlying basis for Schulte *et al.*’s claim that the Chicxulub impact is the sole cause for the Cretaceous-Paleogene (K-Pg) mass extinction is the assumption that the iridium (Ir) anomaly at the K-Pg boundary and Chicxulub are the same age. There is no evidence to support this assertion. No Ir anomaly has ever been identified in association with undisputed Chicxulub impact ejecta (impact glass spherules), and no impact spherules have ever been identified in the Ir-enriched K-Pg boundary clay in Mexico or elsewhere (1, 2). In rare deep-sea sites where the Ir anomaly is just above impact spherules, it is due to condensed sedimentation and/or nondeposition.

A Chicxulub impact-generated tsunami is another basic assumption of Schulte *et al.* to account for the impact spherules in late Maastrichtian sediments (including a sandstone complex) in Mexico and Texas. Multiple lines of evidence contradict this assumption and demonstrate long-term deposition before the K-Pg, including burrowed horizons, multiple impact spherule layers separated by limestone, and spherule-rich clasts that indicate the original deposition predates the K-Pg and excludes tsunami deposition (1–4).

Evidence of the pre-K-Pg age of the Chicxulub impact can also be found in sediments above the sandstone complex in Texas and northeastern Mexico and above the impact breccia in the Chicxulub crater. Evidence shows that the K-Pg boundary is not linked to the sandstone complex and impact spherules (1, 2, 4–7).

Evidence that supports the pre-K-Pg age of the Chicxulub impact is also found in the

presence of a spherule layer in late Maastrichtian sediments below the sandstone complex in northeastern Mexico and Texas (2, 4, 8).

Deccan volcanism is dismissed by Schulte *et al.* as much older and of no consequence in the K-Pg mass extinction. Recent Deccan volcanism studies show the contrary (9–11). These studies link the mass extinction with the main phase of Deccan eruptions.

When this evidence is taken into account, it is clear that the massive Chicxulub and Deccan database indicates a long-term multicausal scenario and is inconsistent with the model proposed by Schulte *et al.*

GERTA KELLER,^{1*} THIERRY ADATTE,²
ALFONSO PARDO,³ SUNIL BAJPAI,⁴
ASHU KHOSLA,⁵ BANDANA SAMANT⁶

¹Department of Geosciences, Princeton University, Princeton, NJ 08544, USA. ²Geological and Paleontological Institute, University of Lausanne, CH 1015 Lausanne, Switzerland. ³Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, Carretera Cuarte s/n 22071, Huesca, Spain. ⁴Department of Earth Sciences, Indian Institute of Technology, Roorkee 247 667, Uttarakhand, India. ⁵Center for Advanced Study in Geology, Panjab University, Chandigarh 160014, India. ⁶Department of Geology, Nagpur University, Nagpur 440 001, Maharashtra, India.

*To whom correspondence should be addressed. E-mail: gkeller@princeton.edu

References

1. G. Keller, *Geol. Soc. Amer. Spec. Pap.* **437**, 147 (2008).
2. G. Keller, W. Stinnesbeck, T. Adatte, D. Stueben, *Earth Sci. Rev.* **62**, 327 (2003).
3. A. A. Ekdale, W. Stinnesbeck, *Paleos* **13**, 593 (1998).
4. G. Keller *et al.*, *Earth Planet. Sci. Lett.* **255**, 339 (2007).
5. G. Keller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 3753 (2004).
6. W. C. Ward, G. Keller, W. Stinnesbeck, T. Adatte, *Geology* **23**, 873 (1995).
7. M. L. Prauss, *Paleogeogr. Paleoclimatol. Paleoecol.* **283**, 195 (2009).
8. G. Keller, T. Adatte, A. J. Pardo, J. G. Lopez-Oliva, *J. Geol. Soc. London* **166**, 393 (2009).
9. G. Keller, T. Adatte, S. Gardin, A. Bartolini, S. Bajpai, *Earth Planet. Sci. Lett.* **268**, 293 (2008).
10. A.-L. Chenet, X. Quidelleur, F. Fluteau, V. Courtillot, S. Bajpai, *Earth Planet. Sci. Lett.* **263**, 1 (2007).
11. A.-L. Chenet, F. Fluteau, V. Courtillot, M. Gerard, K. V. Subbarao, *J. Geophys. Res.* **113**, B04101 (2008).

Response

THE LETTERS BY ARCHIBALD *ET AL.*, KELLER *ET AL.*, and Courtillot and Fluteau question our conclusion that the Cretaceous-Paleogene mass extinction was caused by the asteroid impact at Chicxulub. All three Letters stress that Deccan flood basalt volcanism played a major role in the extinction. Keller *et al.* and Archibald *et al.* also mention that climate change was a factor, and Archibald *et al.* point to marine regression as well.

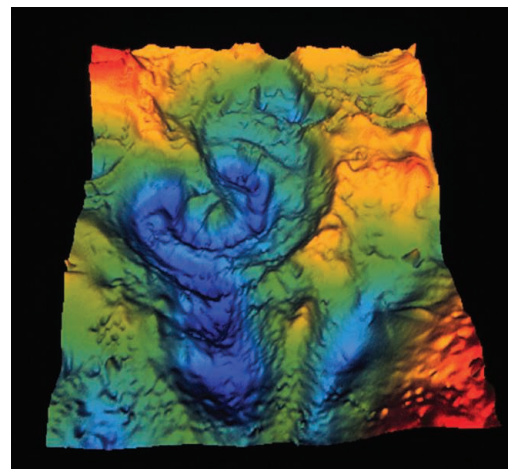
We disagree with the hypothesis that volcanic activity can explain the extinction. First, geographically extensive biotic records of marine microfossils and terrestrial pollen and spores that reveal the nature of the Cretaceous-Paleogene (K-Pg) mass extinction with

the greatest fidelity do not contain evidence of accelerated extinction rates during the last 400 thousand years of the Cretaceous [our Review and (1, 2)] and therefore do not support the idea that the biosphere was somehow destabilized by Deccan volcanism. In fact, plant macrofossils record a diversification during this time (2). Studies of the last 1.5 million years of the Cretaceous from North America, Europe, and Asia [e.g., (3, 4)] are compatible with a sudden extinction scenario for non-avian dinosaurs. Moreover, the constancy of late Maastrichtian open ocean sedimentation (as indicated by climate cycles driven by regular oscillations in Earth's orbit) does not provide evidence for overall declining productivity or instability in marine ecosystems preceding the boundary [e.g., (5)].

Second, recent studies suggest that the emplacement of the Deccan flood basalts took place during multiple (~30) large eruptive pulses, most of which predate the K-Pg boundary by several hundred thousand years (6). In contrast, others have argued that “activity in the continental flood basalt province as a whole is likely to have been quasi-continuous” (7). Nevertheless, it is extremely difficult to reconcile the protracted Deccan flood basalt eruption history with a single abrupt mass extinction horizon exactly at the K-Pg boundary. Although it is well documented that the Chicxulub impact event coincided precisely with sudden paleontological and paleoenvironmental changes and the K-Pg mass extinction [our Review and (8, 9)], there are no comparable data demonstrating that a major pulse of Deccan volcanism coincided with the mass extinction. Moreover, it remains to be explained why one eruptive event would have resulted in mass extinction, whereas multiple earlier eruptive events of comparable magnitude and duration occurring up to 500 thousand years before the K-Pg boundary (6) left few global environmental traces [e.g., (1, 2)].

Third, rates of sulfur injections are critically important to discriminating between environmental consequences of impact versus those of volcanism because the residence time of sulfur in the atmosphere is short (10). Courtillot and Fluteau claim that we misrepresent their 2009 paper (6). However, the paper includes exactly the numbers (reported as “0.1 to 1 Gt/a sulfur dioxide”) we stated. We did not note their finding that the sulfur was released “over durations possibly as short as 100 years for each single eruptive event” (6) because this does not affect our conclusions. Maintaining such a sulfur release for 100 years would indeed result in a total sulfur

release of 50 Gt, which is in the order of the lowest estimate for Chicxulub impact (see our Review). However, sulfur is removed from the atmosphere continuously (10) and therefore any accumulation in the atmosphere is unsupported, contrary to the claim made by Courtillot and Fluteau. We also emphasize that the instantaneous release of 100 to 500 Gt sulfur is only one consequence of the Chicxulub impact, and the K-Pg boundary mass extinction is likely the result of a combination of several impact-induced environmental effects (including the release of sulfur, soot, dust, and other effects, as noted in our Review), whereas the Deccan flood basalt hypothesis relies exclusively on the injection of sulfur dioxide (6).



The Chicxulub Crater. A computer-generated gravity map image shows the Chicxulub Crater on Mexico's Yucatan Peninsula.

With regard to Archibald *et al.*'s and Courtillot and Fluteau's comments about other Phanerozoic mass extinction events that co-occurred with the emplacement of flood basalt provinces, we note that these extinction events are commonly associated with oceanic anoxia, calcification crises, and strong global warming—none of which is observed at the K-Pg boundary (2, 10–13). Furthermore, there is an absence of mass extinctions during several large flood basalt eruptions (10, 14). Each mass extinction event should be considered relative to the record for that event [e.g., (12)], and we stress the unique aspects of the K-Pg boundary record. Chicxulub is by far the largest known impact event in the Phanerozoic, and the projectile hit an extraordinarily thick sulfur-rich sedimentary sequence (see our Review). The absence of evidence for impact phenomena at other mass extinctions, discussed by Archibald *et al.*, is irrelevant for our synthesis of the stratigraphy and biotic response to the specific Chicxulub impact event.

Our work in no way diminishes the importance of gaining a better understanding of the environmental consequences of massive volcanism. We do not doubt that such volcanism can significantly perturb the global environment. However, a robust correlation between mass extinction and flood basalt volcanism as suggested by Courtillot and Fluteau is unlikely [see reviews of (10, 14)].

Keller *et al.* and Archibald *et al.* mention that climate change contributed to the extinction. As outlined in our Review and in (1, 2), climate fluctuations during the latest Maastrichtian (minor warming and subsequent cooling) and the associated faunal and floral consequences are clearly separated from the abrupt mass extinction event at the K-Pg boundary.

In response to Archibald *et al.*'s point about marine regressions, we note that marine mass extinctions may have coincided with global sea-level changes [e.g., (15)]. However, because sea-level changes are numerous (15), this association seems coincidental rather than causal (16). Sea-level change also fails to explain the disruption of vegetation and the faunal change observed in terrestrial environments at the K-Pg boundary (1).

We disagree with the comments of Keller *et al.* regarding the association between Chicxulub impact ejecta and the K-Pg boundary, and we point out that our Review addressed all of the issues to which they refer. Our Review integrated new data with previous work in the peer-reviewed literature to provide substantial corroborating evidence for a global correlation of the Chicxulub impact with the K-Pg boundary.

PETER SCHULTE,^{1*} LAIA ALEGRET,² IGNACIO ARENILLAS,² JOSÉ A. ARZ,² PENNY J. BARTON,³ PAUL R. BOWN,⁴ TIMOTHY J. BRALOWER,⁵ GAIL L. CHRISTESON,⁶ PHILIPPE CLAEYS,⁷ CHARLES S. COCKELL,⁸ GARETH S. COLLINS,⁹ ALEXANDER DEUTSCH,¹⁰ TAMARA J. GOLDIN,¹¹ KAZUHISA GOTO,¹² JOSÉ M. GRAJALES-NISHIMURA,¹³ RICHARD A. F. GRIEVE,¹⁴ SEAN P. S. GULICK,⁶ KIRK R. JOHNSON,¹⁵ WOLFGANG KIESSLING,¹⁶ CHRISTIAN KOEBERL,¹¹ DAVID A. KRING,¹⁷ KENNETH G. MACLEOD,¹⁸ TAKAFUMI MATSUI,¹² JAY MELOSH,¹⁹ ALESSANDRO MONTANARI,²⁰ JOANNA V. MORGAN,⁹ CLIVE R. NEAL,²¹ RICHARD D. NORRIS,²² ELISABETTA PIERAZZO,²³ GREG RAVIZZA,²⁴ MARIO REBOLLEDO-VIEYRA,²⁵ WOLF UWE REIMOLD,¹⁶ ERIC ROBIN,²⁶ TOBIAS SALGE,²⁷ ROBERT P. SPEIJER,²⁸ ARTHUR R. SWEET,²⁹ JAIME URRUTIA-FUCUGAUCHI,³⁰ VIVI VAJDA,³¹ MICHAEL T. WHALEN,³² PI S. WILLUMSEN³¹

¹GeoZentrum Nordbayern, Universität Erlangen-Nürnberg, Schlossgarten 5, D-91054 Erlangen, Germany. ²Departamento de Ciencias de la Tierra e Instituto Universitario de Investigación de Ciencias Ambientales de Aragón, Universidad de Zaragoza, Pedro Cerbuna 12, E-50009 Zaragoza, Spain. ³Department of Earth Sciences, University of Cam-

bridge, Cambridge CB2 3EQ, UK. ⁴Department of Earth Sciences, University College London, Gower Street, London WC1E 6BT, UK. ⁵Department of Geosciences, Pennsylvania State University, University Park, PA 16802, USA. ⁶Institute for Geophysics, Jackson School of Geosciences, University of Texas at Austin, Austin, TX 78759, USA. ⁷Earth System Science, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium. ⁸Centre for Earth, Planetary, Space, and Astronomical Research, Open University, Milton Keynes, MK7 6AA, UK. ⁹Earth Science and Engineering, Imperial College London, London SW7 2BP, UK. ¹⁰Institut für Planetologie, Universität Münster, D-48149 Münster, Germany. ¹¹Department of Lithospheric Research, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria. ¹²Planetary Exploration Research Center, Chiba Institute of Technology 2-17-1 Tsudanuma, Narashino, 275-0016 Chiba, Japan. ¹³Programa de Geologia de Exploración y Explotación, Dirección de Investigación y Posgrado, Instituto Mexicano del Petróleo, Eje Lázaro Cárdenas No. 152, C.P. 07730, México City, México. ¹⁴Earth Sciences Sector, Natural Resources Canada, Ottawa, ON K1A 0E4, Canada. ¹⁵Research and Collections Division, Denver Museum of Nature and Science, 2001 Colorado Boulevard, Denver, CO 80205, USA. ¹⁶Museum für Naturkunde, Leibniz Institute at the Humboldt University Berlin, Invalidenstrasse 43, D-10115 Berlin, Germany. ¹⁷Center for Lunar Science and Exploration, Universities Space Research Association-Lunar and Planetary Institute, Houston, TX 77058-1113, USA. ¹⁸Department of Geological Sciences, University of Missouri, Columbia, MO 65211, USA. ¹⁹Earth and Atmospheric Sciences, Purdue University, West Lafayette, IN 47907-2051, USA. ²⁰Osservatorio Geologico di Coldigioco, 62021 Apiro (MC), Italy. ²¹Department of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA. ²²SIO Geological Collections, Scripps Institution of Oceanography, La Jolla, CA 92093-0244, USA. ²³Planetary Science Institute, Tucson, AZ 85719, USA. ²⁴Department of Geology and Geophysics, School of Ocean and Earth Science and Technology, University of Hawaii, Manoa, Honolulu, HI 96822, USA. ²⁵Unidad de Ciencias del Agua, Centro de Investigación Científica de Yucatán, Cancún, Quintana Roo, 77500, México. ²⁶Laboratoire des Sciences du Climat et de l'Environnement, Institut Pierre et Simon Laplace, Commission à l'Énergie Atomique/CNRS/Université de Versailles Saint Quentin en Yvelines-UMR 1572, Avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex, France. ²⁷Brüker Nano GmbH, Schwarzschildstraße 12, D-12489 Berlin, Germany. ²⁸Department of Earth and Environmental Sciences, K.U.Leuven, Box 2408, Celestijnenlaan 200E, 3001 Leuven, Belgium. ²⁹Natural Resources Canada, Geological Survey of Canada Calgary, 3303 33rd Street NW, Calgary, AB T2L 2A7, Canada. ³⁰Laboratorio de Paleomagnetismo y Paleoambientes, Programa Universitario de Perforaciones en Océanos y Continentes, Instituto de Geofísica, Universidad Nacional Autónoma de México (UNAM), DF 04510 México, México. ³¹Department of Earth and Ecosystem Sciences, Lund University, Sölvegatan 12, 223 62 Lund, Sweden. ³²Department of Geology and Geophysics, University of Alaska, Fairbanks, AK 99775, USA.

*To whom correspondence should be addressed. E-mail: schulte@geol.uni-erlangen.de

References

- D. J. Nichols, K. R. Johnson, *Plants and the K-T Boundary* (Cambridge Univ. Press, 2008).
- P. Wilf, K. R. Johnson, B. T. Huber, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 599 (2003).
- D. E. Fastovsky, P. M. Sheehan, *GSA Today* **15**, 4 (2005).
- V. Riera, O. Oms, R. Gaete, A. Galobart, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **283**, 160 (2009).
- T. D. Herbert, I. Premoli-Silva, E. Erba, A. G. Fischer, *Soc. Econ. Paleont. Mineral. Spec. Publ.* **54**, 81 (1995).
- A.-L. Chenet *et al.*, *J. Geophys. Res.* **114**, B06103 (2009).
- A. E. Jay *et al.*, *J. Geol. Soc.* **166**, 13 (2009).
- T. J. Bralower *et al.*, *Geology* **38**, 199 (2010).
- S. Jiang *et al.*, *Nat. Geosci.* **3**, 280 (2010).
- P. B. Wignall, *Earth Sci. Rev.* **53**, 1 (2001).
- R. V. White, A. D. Saunders, *Lithos* **79**, 299 (2005).
- V. Vajda, S. McLoughlin, *Rev. Palaeobot. Palynol.* **144**, 99 (2007).
- W. Kiessling, C. Simpson, *Global Change Biol.*, published online 24 February 2010; 10.1111/j.1365-2486.2010.

02204.x, in press.

- S. Kelley, *J. Geol. Soc. London* **164**, 923 (2007).
- K. G. Miller *et al.*, *Science* **310**, 1293 (2005).
- A. B. Smith, A. S. Gale, N. E. A. Monks, *Paleobiology* **27**, 241 (2001).

Honing the Test-and-Treat HIV Strategy

IN HIS NEWS FOCUS STORY ("TREATMENT AS prevention," 5 March, p. 1196), J. Cohen reviews ideas presented at the 17th Conference on Retroviruses and Opportunistic Infections about the use of HIV treatment as prevention. Enthusiasm for the treatment-as-prevention approach has grown in recent years as (i) the drugs have become safer, better tolerated, and more widely available; (ii) widespread testing has become cheaper and more efficient; (iii) earlier therapy has become desirable; and (iv) mathematical modeling by some (1) (but by no means all) has suggested that a test-and-treat strategy could control the spread of HIV.

Cohen cites an observational analysis, by Donnell *et al.*, that reported considerable reduction of HIV transmission in HIV discordant couples when ART was provided to the HIV-infected index partner (2). This finding—similar to work from Sullivan *et al.* presented at Conference on Retroviruses and Opportunistic Infections in 2009 (3)—helps to support the key assumption that ART reduces infectiousness. However, these studies report only short-term observations; they do not address the durability of this effect or the risk of transmitted drug-resistant HIV strains, two critical considerations for the test-and-treat strategy.

TIMOTHY D. MASTRO^{1*} AND MYRON S. COHEN²

¹Family Health International, Research Triangle Park, NC 27709, USA. ²Division of Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.

*To whom correspondence should be addressed. E-mail: tmastro@fhi.org

References

- R. M. Granich, C. F. Gilks, C. Dye, K. M. De Cock, B. G. Williams, *Lancet* **373**, 48 (2009).
- D. Donnell *et al.*, abstract 136, presented at the 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, 16 to 19 February 2010.
- P. Sullivan *et al.*, abstract 52bLB, presented at the 16th Conference on Retroviruses and Opportunistic Infections, Montreal, Canada, 8 to 11 February 2009.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

SOCIAL SCIENCES

Selfish Games

David Krakauer

The paradoxical seriousness of games was reviewed synoptically by the Dutch historian Johan Huizinga in his 1938 book *Homo Ludens* (1). It includes a description of a community of states whose “diplomatic forms, its mutual obligations in the matter of honoring treaties ... in the event of war ... all bear a formal resemblance to play-rules inasmuch as they are only binding while the game itself ... is recognized.”

In 1944, John von Neumann and Oskar Morgenstern published *Theory of Games and Economic Behavior* (2). This monograph consolidated mathematical game theory and provided fortuitous support for the humanistic insights of Huizinga. Although the essential ideas for game theory grew out of the analysis of parlor games, they were quickly generalized to matters as serious as the chilling cold war strategy of mutually assured destruction.

Game theory provides a mathematical tool kit for analyzing strategic interactions by assigning payoff values (utility values) to sets of individual strategies and seeks to determine strategy stability. The best-known stability criterion is the Nash equilibrium, which defines pairs of strategies from which any deviation will reduce the payoff.

In *The Calculus of Selfishness*, Karl Sigmund provides a comprehensive and accessible mathematical exposition of the evolutionary game theory of selfishness. The book should prove accessible to natural and social scientists as its mathematical arguments employ intuition, geometry, and simulation with a minimum of axiomatic formality. The demands on the reader typically involve little more than linear algebra and calculus.

For Sigmund (a mathematician at the University of Vienna), in the spirit of Adam Smith, selfishness implies the enlightened self-interest of individuals. The problem that game theory seeks to explain is reciprocity among selfish individuals. After opening the book with a short history, Sigmund structures it through a sequence of games. The first is the prototype of reciprocal gaming, the prisoner's dilemma. In subsequent chapters, he

includes player identity and memory, allowing for indirect reciprocity. He then introduces the ultimatum game and, finally, public goods games. This hierarchy of games corresponds to an expansion of the volume of social concepts to include fairness, trust, incentives, sanctions, and moral sentiments.

Sigmund's preferred approach to game theory is to consider a well-mixed population of players, with those obtaining the highest payoffs preferentially replicating or imitated. Over time, these strategies increase in frequency at the expense of

others. This is an evolutionary approach to game theory that replaces rational deliberation by individuals with a Darwinian population dynamic. At the center of this approach is replication, typically modeled in deterministic systems by using the replicator equation. Some of the more technical elements in the book are devoted to the analysis of this equation and its derivation through imitation processes. The key insights supporting the use of dynamics are that the saturated rest points of

the replicator equation correspond to Nash equilibria and that, through Brouwer's fixed point theorem, all symmetric games possess Nash solutions.

The author's style of model building is incremental. He adds spatial and temporal dimensions to an initial one-shot, bimatrix game. His simplest game has two dimensions of strategy, memory for one round, and one shot of play (2D, 1M, 1T). He builds the models up to include more strategies, increased memory depth, and iterated rounds of play: (2D, 1M, 1T), (2D, 1M, m T), (2D, n M, 1T), ... (n D, n M, m T). He also introduces errors to allow for a small probability that the wrong strategy is adopted, and he analyzes continuous strategy spaces in an adaptive dynamics setting. This makes for a lucid communication of ideas grounded on a foundational dynamical system.

Having reviewed methods, what of concepts? I consider the three chapters on reputation, fairness and trust, and public goods the richest in new insights. These chapters provide the strongest evidence for the ability of simple mathematical ideas to illuminate complex psychological and social phenomena. Moreover, these models are often of greatest value when they fail in interesting ways to account for the results of experimental economics studies, suggesting either a flaw in assumptions or insufficient complexity of the models. Such failures are interesting because the models tend to bias solutions in the direction of individual, short-sighted outcomes coupled to low payoffs.

These model-data discrepancies appear frequently in studies of indirect reciprocity and in experimental game-based studies of fairness. Indirect reciprocity arises in repeated games where one's behavior is directed against individuals with whom one did not interact in a previous round. Vicarious reciprocity describes the case where altruistic behavior is directed toward an altruist with whom one did not interact in a previous round. Misguided reciprocity is altruistic behavior directed toward a recipient of altruism in a previous round. Models can generate robust vicari-

The Calculus of Selfishness

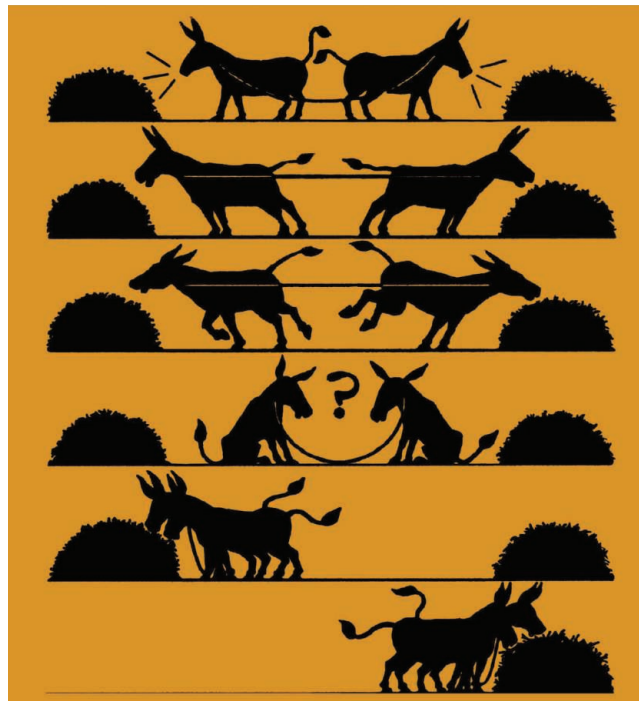
by Karl Sigmund

Princeton University Press,
Princeton, NJ, 2010.

184 pp. \$35, £24.95.

ISBN 9780691142753.

Theoretical and Computational Biology.



The Two Mules.

The reviewer is at the Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501, USA. E-mail: krakauer@santafe.edu

ous reciprocity but not misguided reciprocity, which is a common feature in the experimental data on human subjects.

Similar anomalies are observed in models of the ultimatum game in which a proposer offers a fraction of her wealth to a responder, who can accept the sum or reject it (in which case both parties receive nothing). Simple models discover Nash solutions with the minimum offer, whereas the data frequently reveal fair offers (e.g., 50%). In this case, augmenting the models to allow for reputation effects can restore fairness to the game solutions.

The Calculus of Selfishness offers a valuable review of recent progress in evolutionary approaches to prosocial phenomena. In a world where selfishness has been called the defining human characteristic, we need all the thoughtful help we can get.

References

1. J. Huizinga, *Homo ludens: Proeve eener Bepaling van Het Spelelement der Cultuur* (Tjeenk Willink, Haarlem, Netherlands, 1938).
2. J. von Neumann, O. Morgenstern, *Theory of Games and Economic Behavior* (Princeton Univ. Press, Princeton, NJ, 1944).

10.1126/science.1189117

ECONOMICS

Identity Matters

Robert Sugden

Until quite recently, most economic theories were based on a priori assumptions about the rationality of individuals' choices. Much of the most prestigious work in economic theory was directed at refining the concept of rationality, developing rational-choice explanations for what had previously been seen simply as reliable empirical regularities, and extending the scope of rational-choice theory to colonize the domains of other social sciences. Now, however, there is a move toward the relaxation of rationality assumptions and the import of explanatory principles from other disciplines. Game theorists are borrowing modeling techniques from theoretical biology, explaining human decisions as the result of trial-and-error learning or of blind processes of selection. Behavioral economists are drawing on the ideas and

experimental methods of cognitive psychology and neuroscience. George Akerlof and Rachel Kranton's *Identity Economics* opens a new front in this transformation of economics by incorporating ideas from sociology and anthropology. Given economists' traditional suspicion of these disciplines as lacking in theoretical rigor, this is a particularly brave move.

The key concept that Akerlof and Kranton (economists at, respectively, the University of California, Berkeley, and Duke University) take from sociology is identity. Societies are structured by commonly understood categorizations (such as those of age, class, race, and gender) into groups with their own norms of behavior. Understanding their identities in terms of these categories, individuals feel pressure to follow the corresponding norms. Akerlof and Kranton translate this idea into economic language by postulating that individuals gain (or lose) "identity utility" by conforming to (or deviating from) the norms of their identities.

The authors apply the idea of identity to four issues having important economic dimensions: incentives and motivation in the workplace, motivation to learn in schools, gender asymmetries in labor markets, and interactions between race and poverty. Akerlof and Kranton review a wide range of ethnographic studies that demonstrate the importance of identity. Take the case of gender. Many occupations and modes of working are still stereotyped as male or female. A female corporate lawyer who is ambitious and forceful is perceived by her workmates as unfeminine; a male nurse who is sympathetic and caring is perceived as unmasculine. Women who enter male-dominated occupations are systematically harassed by their male co-workers. Akerlof and Kranton argue convincingly that individuals' desires to maintain their gender identities are crucial for the reproduction of gender asymmetries. They conclude that public policy should aim to shift perceptions of the norms associated with identity. Thus, they give the women's movement much of the credit for recent reductions in the extent of gender asymmetries in the labor market and highlight the significance of gender-neutral job titles (such as "firefighter" rather than "fireman"). Similarly, they commend African-American leaders who have sought to "change what it means to be black."

The main analytical work in these stud-

ies is sociological and ethnographical. It is by consulting the findings of researchers who have used the methods of these disciplines that Akerlof and Kranton discover which categories are socially recognized, what norms are associated with each identity, and how perceptions of identity can be influenced by policy. So it is surprising that, when urging their fellow economists to adopt new methodologies, the authors' main

emphasis is on representing identity in economic models. They acknowledge that the results of their models are often not particularly surprising, once the sociological ingredients are understood: "What is new ... is the assumptions." But they seem reluctant to propose that economists do the work necessary to find which assumptions are

required. Just as behavioral economists have learned to run the sorts of experiments that once were the preserve of psychologists, one might have thought that identity economists would learn to write ethnographies. But perhaps that would be taking bravery too far.

Identity Economics is a popular account of work that will already be familiar to economists who have read the authors' journal articles. It is admirably short, written in a clear, nontechnical style but without the condescending breeziness of many books aimed at the airport market. Nonspecialist readers will find a lot of insightful and well-informed analysis of how issues of identity have an impact on real economic problems. However, these readers may be bemused by Akerlof and Kranton's descriptions of their models and discussions of their general modeling strategy. The authors defend this strategy on the grounds that it generates more satisfactory explanations of economic reality than traditional economic theory can supply. But their presentations of the models themselves are so brief and nontechnical that the reader ends up having to take that claim on trust. Presumably, Akerlof and Kranton judged that their readers would be bored by more detailed discussion or would not understand it. But then why are the same readers expected to be interested in, or able to evaluate, the relative merits of different modeling strategies in economics? One sometimes has the sense that arguments that really are addressed to fellow economists are being played out in front of a general audience.

Identity Economics
How Our Identities
Shape Our Work, Wages,
and Well-Being
by George A. Akerlof
and Rachel E. Kranton
Princeton University Press,
Princeton, NJ, 2010.
191 pp. \$24.95, £16.95.
ISBN 9780691146485.

10.1126/science.1189285

The reviewer is at the School of Economics, University of East Anglia, Norwich NR4 7TJ, UK. E-mail: r.sugden@uea.ac.uk

SCIENCE AND SOCIETY

The Smart Electricity Grid and Scientific Research

Jan Beyea

So-called “smart” meters and appliances have the potential to save energy, to shave peak electricity usage, and to reduce risks of blackouts (1–6). Typical smart meter designs include periodic transmission of current, phase, and frequency data from the user to the electricity distribution company. Utilities will use the data in billing calculations under time-of-day pricing, for load-management research, to provide customer feedback, and/or to adjust customer appliances.

However, there has been little discussion of the full scientific, economic, and historic potential of these data, whose usefulness may extend beyond the original purposes for which the data will be collected and stored. Input is needed from third-party researchers (neither customers nor utilities) at this early and critical stage of the smart grid’s development, with legislation and regulations yet to come. Otherwise, it will be hard for regulators to reach a balanced decision on which high-resolution data should be stored, for how long, and to whom it should be made accessible inside and outside the collecting utility.

How Much Data?

The Obama Administration is projecting 40 million smart meters in the United States “over the next few years” (7). The European Union is planning to install 245 million within the next 10 years (8, 9). Some systems in Europe are already transmitting at 1-s intervals (10). Computer algorithms are capable of converting 1-s interval data into a timeline of smart appliance use (6, 11–14) by making use of libraries of characteristic appliance signals; performance degrades as the time resolution becomes coarser (11).

The cost of storing a customer’s data would be trivial compared with a customer’s annual electricity bill, even without data compression. A stream of four numbers (current, phase, frequency, and time) transmitted every second amounts to 0.5 gigabytes of data per year per customer, about as much data as are stored on one standard compact disc. The data from 100 million customers, roughly

the amount of households in the United States, would amount to 50 petabytes per year before compression. About 0.05 petabytes would be needed to store data at 15-min intervals, reflective of many first-generation smart meters. The sample sizes potentially available for research are huge, but manageable, even without subsampling. Data sets in the multipetabyte range are already being analyzed in biology and physics (15). For example, about 2.5 petabytes of data are stored in mammograms each year in the United States (15). About 15 petabytes are projected to be stored each year at the Large Hadron Collider (16).

Privacy

Privacy protection is a major challenge for smart meter systems and a challenge for any use of social-spatial data by the scientific community at large, because it is possible to use high-resolution data to infer personal habits. The implications related to unwarranted government searches under the Fourth Amendment of the U.S. Constitution are sobering (11, 17), and privacy guidelines are being developed and debated (18–20).

But if privacy-protecting access techniques are approved by regulators for energy management purposes, some variant should merit approval for use in third-party research. For instance, data may be confined to utility servers, with analysis algorithms transmitted from researchers and executed on the server. Also, many researchers have great experience dealing with the privacy of study subjects through service on, and interactions with, Institutional Review Boards (21, 22). Consent could be obtained, just as it is in any human-subject study involving individualized data. However, some privacy risks will always remain (20), and some data will have to be off limits.

Promising Uses of Data

Even data that are stripped of all recorded personal identifiers to minimize the risk of privacy invasion (de-identified data) could

Researchers have an interest in access to, and preservation of, data that will soon be collected by the smart electricity grid.



help answer questions of interest to energy researchers and social scientists. Are there ways to spot impending electricity outages? How does energy usage correlate with current events, appliance standards, and price? Which utility programs work best to improve energy efficiency? How are appliance efficiencies changing over time? How varied is the usage of appliances from person to person, from region to region, and from decade to decade?

Even more useful would be de-identified data that retain some geographic information, such as county, town, census tract, and/or census block. Privacy risks would be minimal, and demographic data collected in the census, as well as local meteorological data, could be added to the electricity data set, thereby increasing its usefulness for group-level analysis. Economists and energy modelers could use the combined data to examine how electricity usage—even appliance by appliance, in some cases—is related to price, average household income, and average family size, as well as other aggregated variables available in census block data. The simultaneous responses of millions of customers to episodic weather changes, national tragedies, presidential proclamations, changes in laws,

and cultural trends could be studied by social scientists. Comparisons across countries could be fascinating.

Electricity use in the home is of interest to epidemiologists. Appliance usage may be a main effect variable in epidemiologic studies of health impacts of electromagnetic fields (23). It may be a confounding variable in studies of air pollution or other causes of disease (24). Smart meters could provide direct data on use of some appliances (e.g., microwaves and electric blankets), as well as a check on usage data for other appliances obtained from the questionnaires normally used in such studies.

Other types of health studies (e.g., intervention studies) can benefit from access to detailed, individualized electricity data. Algorithms might be designed, for example, to infer how many times per day a refrigerator door was opened (relevant to dietary and obesity studies), the times of day a residence was occupied (relevant to air pollution health studies), and even the pattern of sleep (relevant to a variety of health studies).

Some uses of data may be unknown today, but important decades hence. Saved electricity data may help future generations solve unanticipated problems, just as 1960s satellite images collected before awareness of the climate problem assist us today (25).

Data Ownership and Access

Individual user data are considered to belong either to the customer or to the collecting utility, with access to the data in aggregate limited to the utility, subcontractors, and third-party energy-management companies. But other third-party researchers, who do not yet appear to be viewed as major potential users of data from smart meters, need to be added to the picture; otherwise, privacy regulations might inadvertently freeze them out (1–6, 8, 11, 17, 26). Even if access were expanded to include such outside researchers—under as yet undetermined restrictions—it is common practice for utilities to destroy customer data after a delay period chosen to allow for long billing disputes. This delay is often 7 years in the United States (17). This means that data would be lost to historians, unless specific regulations were implemented to allow data, or a subset of them, to be transferred to an archival repository.

How might a research project get approval to access data? A review board or staff at a utility commission might handle the duties of performing due-diligence assessments of research requests, working in consort with the Institutional Review Boards at the requesters' institutions. Funding of the proj-

ect by government agencies that used a peer-review grant process might be a requirement that would ensure, for example, that sample size, measurement error, and other issues have been accounted for in any proposed use of specific appliance data.

Electricity data are only one example where the government has bargaining power (e.g., via public utility commissions) to facilitate access to data by qualified researchers, but taking action is timely, because this data revolution is in its very early stages. In addition, tax benefits may soon be given for installation of smart meter systems; sharing of data (with privacy protection mandated) could be made a prerequisite.

Furthermore, it is the customers as a group who will pay for the meters. As part of the “regulatory bargain” (27) that gives government control of prices, profitability, and service standards of monopoly utility companies, regulators will allow the electricity distribution company (a monopoly) to pass on direct costs, raise necessary capital, and make a limited, but guaranteed, rate of return on prudent investments. Under such arrangements, regulators could include (modest) costs needed to facilitate third-party research.

Planning for the Future

Now is the time for researchers to join discussions about data from smart meters. The National Institute of Standards and Technology is developing model standards in the United States for information management of smart grid devices (www.nist.gov/smartgrid/). The European Commission has a Smart Meter Coordination Group (www.cen.eu). Contact with state and local electricity regulators could be fruitful. Many utility companies will likely be helpful, if regulators consent to third-party research. Studies could be carried out in collaboration with industry research institutions such as the Electric Power Research Institute. Some utilities, however, may recoil at outside research, for example, on health effects from electromagnetic fields, because of concerns about possible future liability and/or bad publicity. Large samples can turn up associations that are very weak, but still statistically significant, which might be problematic for companies, depending on the presentation of results.

At this moment of transition for the electrical grid, researchers and their institutions can work with local utilities and local regulators, and scientific societies can work at the national level, to present the case for the benefits of access and research, to describe scientists' experience with privacy protection, and to help make sure that third-

party researchers are not excluded by rules set without considering them. Reviews of third-party research concepts by a National Research Council panel that included privacy scholars would be useful, as would reviews by equivalent bodies outside the United States. Their reviews and recommendations could consider other massive data sets—e.g., cell-phone geospatial data, whose access might also be made feasible as part of regulatory and legislative bargains.

References

1. R. J. Meyers, E. D. Williams, H. S. Matthews, *Energy Build.* **42**, 563 (2010).
2. A. Faruqi, R. Hledik, S. Sergici, *Electr. J.* **22**, 55 (2009).
3. G. Strbac, *Energy Policy* **36**, 4419 (2008).
4. S. Roberts, *Energy Policy* **36**, 4552 (2008).
5. M. J. King, K. King, M. B. Rosenzweig, *Electr. J.* **20**, 38 (2007).
6. M. Newborough, P. Augood, *IEE Proc.* **146**, 283 (1999).
7. White House, *President Obama Announces \$3.4 Billion Investment to Spur Transition to Smart Energy Grid* (White House, Washington, DC, 2009); www.whitehouse.gov/the-press-office/president-obama-announces-34-billion-investment-spur-transition-smart-energy-grid.
8. M. Venables, *Eng. Technol.* **4**, 10 (2009).
9. M. A. Lisovich, S. B. Wicker, *IEEE Proc. Power Syst.* **1**, 1 (2008).
10. M. K. El Mahrsi, S. Vignes, G. Hebrail, M.-L. Picard, *Proceedings of the Third International Conference on Research Challenges in Information Science* (RCIS 2009), Fez, Morocco, 22 to 24 April 2009 (IEEE, New York, 2009), pp. 395–402; <http://ieeexplore.ieee.org/iel5/5073917/5089258/5089303.pdf%3Farnumber%3D5089303&authDecision=203>.
11. M. A. Lisovich, S. B. Wicker, *IEEE Proc. Power Syst.* **1**, 1 (2008).
12. A. Prudenzi, *IEEE Power Eng. Soc. Winter Meeting* **2**, 941 (2002).
13. S. Drenker, A. Kader, *Comput. Appl. Power IEEE* **12**, 47 (1999).
14. F. Sultanem, *IEEE Trans. Power Deliv.* **6**, 1380 (1991).
15. A. J. G. Hey, A. E. Trefethen, in *Grid Computing: Making the Global Infrastructure a Reality*, F. Berman, G. C. Fox, A. J. G. Hey, Eds. (Wiley, Hoboken, NJ, 2003), pp. 809–824.
16. The European Organization for Nuclear Research (CERN), *Worldwide LHC Computing Grid*; <http://public.web.cern.ch/public/en/LHC/Computing-en.html>.
17. J. I. Lerner, D. K. Mulligan, *Stanford Technol. Law. Rev.* **2008**, 3 (2008).
18. U.S. National Institute of Standards and Technology (NIST), *Smart Grid Cyber Security Strategy and Requirements* (Draft NISTIR 7628, NIST, Gaithersburg, MD, 2009); <http://csrc.nist.gov/publications/drafts/nistir-7628/draft-nistir-7628.pdf>.
19. European Committee for Standardization (CEN), *Workshop on Data Protection and Privacy*; www.cen.eu/cenorm/sectors/sectors/iss/activity/wsdpp.asp.
20. D. Lazer et al., *Science* **323**, 721 (2009).
21. T. W. Rice, *Respir. Care* **53**, 1325 (2008).
22. K. B. Enfield, J. D. Truitt, *Respir. Care* **53**, 1330 (2008).
23. G. Mezei, M. Gadallah, L. Kheifets, *Epidemiology* **19**, 424 (2008).
24. M. D. Gammon et al., *Breast Cancer Res. Treat.* **74**, 235 (2002).
25. H. Pringle, *Science* **327**, 1322 (2010).
26. Office of Science and Technology Policy Forum, *Consumer Interface with the Smart Grid Blog*, <http://collaborate.nist.gov/wiki-sggrid/bin/view/SmartGrid/OSTPBlogWeek2>.
27. D. N. Jones, *Energy Law J.* **22**, 41 (2001).

10.1126/science.1189229

MICROBIOLOGY

Salmonella's Safety Catch

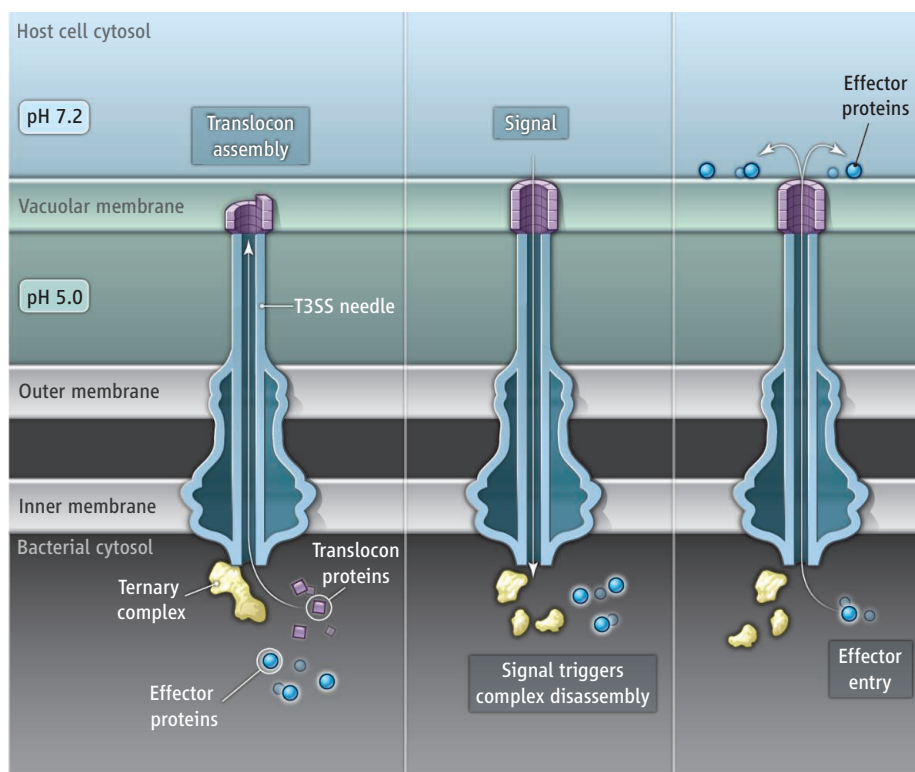
R. John Collier

Many pathogenic bacteria disable their host's immune system and thereby protect themselves from destruction by delivering "effector proteins" into the inner, cytosolic compartment of the eukaryotic host cells. Gram-negative bacteria, in particular, have evolved an elaborate "needle complex"—the type III secretion system (T3SS), consisting of more than 20 proteins—to convey an arsenal of effector proteins from their own cytosolic compartment across three membranes directly into the cytosol of the host cell (1). These bacteria spend much of their lives in transit between eukaryotic hosts, however, and it would be a potentially catastrophic waste of their resources to secrete effector proteins continuously into their environment. On page 1040 of this issue, Yu *et al.* (2) describe a system to regulate the secretion process in *Salmonella enterica*, an important intracellular pathogen of humans and animals.

The authors show that after the bacterium enters the host cell within a membrane-bound vacuole, it detects its arrival there by sensing the pH sequentially in two locations: first the acidic pH within the vacuole and then, once the T3SS has penetrated the vacuolar membrane, the neutral pH of the cytosol outside the vacuole. Only then does the bacterium allow its effector proteins to proceed through the T3SS needle into the host cell cytosol to control selected metabolic processes of that cell.

The T3SS needle complex has at its base a multiring substructure with 19- to 22-fold rotational symmetry. This substructure spans the inner and outer membranes of the bacterial envelope and serves as the fundamental unit for secretion. The base secretes three classes of proteins: subunits of a narrow needle, which is connected to the base and extends outward from the cell surface; translocon proteins, located at the tip of the needle and capable of forming a pore in a host cell membrane; and effector proteins, which pass through the needle and the translocon pore into the cytosol of the host cell.

The regulatory system described by Yu *et al.* consists of three proteins—SsaL, SsaM, and SpiC—thought to be located at or near the portal through which proteins enter the secretion channel in the base of the T3SS needle



Regulation of the *S. enterica* secretion process. (Left) After *S. enterica* has entered a host cell, the acidity of the vacuole within which the bacterium resides induces assembly of the T3SS needle complex. A regulatory complex (yellow) formed from SsaL, SsaM, and SpiC at the entry portal of the needle prevents secretion of effectors (blue), but allows translocon proteins (purple) to enter and proceed to the tip of the needle, where they form a pore in the vacuolar membrane. (Middle) The pore enables the elevated pH of the host cell cytosol to be sensed, causing the disassembly of the SsaL/SsaM/SpiC (ternary) complex. (Right) Effector proteins can now pass through the needle complex and enter the cytosol of the host cell.

complex. The three proteins form a ternary complex when the bacteria are grown at pH 5.0, an acidity that mimics the environment in the vacuole and is required for functional assembly of the T3SS (3). An intact ternary complex is required for secretion of translocon proteins and at the same time suppresses effector secretion, which suggests that it forms a selective gateway to the channel.

When Yu *et al.* exposed wild-type bacteria to a neutral-pH medium after they had been grown in an acidic medium, they found that effector secretion was raised by an order of magnitude, indicating two sequential steps of pH sensing. Under the assumption that translocon proteins at the tip of the T3SS needle insert into the surrounding vacuolar membrane in an infected cell, one can readily conceive that the needle could provide a pathway for sensing the neutral pH outside the vacuole. Indeed, when the authors lowered the pH

of the cytosol of *S. enterica*-infected cells from neutral to acidic, effector secretion was suppressed. These results suggest that the pH of the host cell cytosol is a signal for shifting the secretion process from translocon proteins to effector proteins.

How is the integrity of the ternary complex affected by exposing bacteria grown at pH 5.0 to a pH 7.2 medium? As the authors show, the increase in pH leads to dissociation of the complex. Further, levels of SsaL and SsaM declined, and the decline was delayed in infected cells with acidified cytosolic pH or lacking a translocon pore. Taken together, the results indicate that the regulatory complex acts as a selective gate, which dissociates following translocon-dependent sensing of host cytosolic pH, relieving the suppression of effector secretion (see the figure).

What is the nature of the pH sensor that induces dissociation of the ternary regula-

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA. E-mail: john_collier@hms.harvard.edu

CREDIT: Y. GREENMAN/SCIENCE

tory complex? Changes in the concentrations of specific ions, in particular H^+ and Ca^{2+} , can induce major changes in conformation of some proteins. A relevant example is the pH-dependent conformational transition that enables some bacterial toxins (such as diphtheria and anthrax toxins) to insert into endosomal membranes, form pores, and translocate their effector subunits to the cytosol under acidic conditions (4). In these cases, the sensor protein is the toxin itself. In T3SS, one candidate is the needle subunit, which may undergo a conformational shift that

is transmitted to the regulatory complex.

The work by Yu *et al.* presents an intriguing model of how *S. enterica* senses contact with the host and how it reacts to a signal from another compartment (the cytosol) from that in which it replicates. It is currently unknown whether pH differentials, as described here, are a widespread means of host sensing by other intracellular Gram-negative bacteria as well, but there is evidence from studies with *Yersinia* and *Escherichia coli* that the low Ca^{2+} concentration of the host cell may be the basis of a

similar T3SS switch in those organisms (5, 6).

References

1. J. E. Galán, H. Wolf-Watz, *Nature* **444**, 567 (2006).
2. X.-J. Yu, K. McGourty, M. Liu, K. E. Unsworth, D. W. Holden, *Science* **328**, 1040 (2010); published online 15 April 2010 (10.1126/science.1189000).
3. C. Rappl, J. Deiwick, M. Hensel, *FEMS Microbiol. Lett.* **226**, 363 (2003).
4. J. A. Young, R. J. Collier, *Annu. Rev. Biochem.* **76**, 243 (2007).
5. W. Deng *et al.*, *Infect. Immun.* **73**, 2135 (2005).
6. V. T. Lee, S. K. Mazmanian, O. Schneewind, *J. Bacteriol.* **183**, 4970 (2001).

10.1126/science.1190758

GEOPHYSICS

The Enigmatic Inner Core

Bruce A. Buffett

Over the past billion years or so, Earth's liquid iron core has cooled and solidified, resulting in the growth of a solid inner core (1). Seismic data on the structure of this inner core reveal a bewildering degree of complexity (2, 3). Most surprising is the observation that the seismic properties differ between the eastern and the western half of the inner core (see the figure) (4). How could distinct hemispheres develop as the inner core has grown? On page 1014 of this issue, Monnereau *et al.* (5) propose an intriguing answer: They suggest that as the inner core has grown, material has moved laterally within it. When this lateral motion is sufficiently fast, melting and solidification should occur in opposite hemispheres. Newly formed iron crystals in one hemisphere would grow with age during transit across the inner core. The hemispherical differences in seismic properties emerge from differences in average crystal sizes. Further insight into the properties of the inner core is provided by Deuss *et al.* on page 1018 (6), who develop a new method for estimating hemispherical structure.

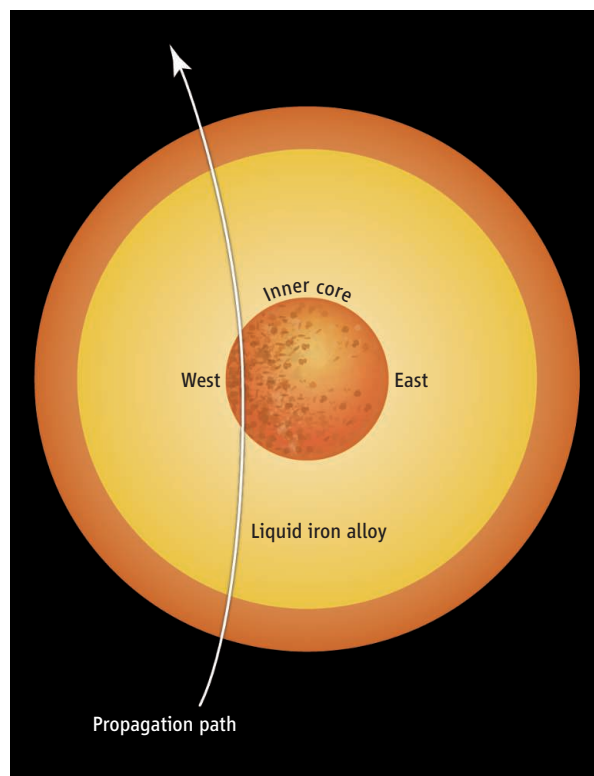
Monnereau *et al.* argue that lateral motion should be expected when the inner core is unstable to thermal convection. The argument runs as follows. Temperature perturbations in one hemisphere alter the density field and shift the center of mass of the inner core. The surface of the inner core is displaced through internal temperature and pressure gradients, causing departures from thermodynamic equilibrium. Melting and

solidification act to return the surface of the inner core to thermodynamic equilibrium, whereas a hydrostatic (mechanical) equilibrium restores the boundary to its displaced position. Steady lateral motion develops as

the center of mass settles into a statically displaced location.

The authors do not attempt to quantify the rate of lateral motion on purely dynamical grounds. Instead, they constrain the motion with seismic observations. Hemispherical differences in seismic wave speed and attenuation have previously been reported in the top 100 km of the inner core (see the figure) (7, 8). Monnereau *et al.* show that variations in crystal size can explain these observations. To match the amplitude of the observed hemispherical variations, the lateral motion must be at least three times as large as the growth rate. If correct, this conclusion offers exciting opportunities to explore the processes that control the rate of lateral motion.

Another surprising feature of the inner core is the presence of seismic anisotropy, which causes wave speeds to depend on the direction of travel (see the figure) (9, 10). Seismic anisotropy is usually attributed to preferential alignment of crystal lattices. Lateral motion of the inner core produces no deformation (and hence no crystal alignment), so we must appeal to other mechanism to explain the presence of seismic



East and west are not the same. Propagation of seismic waves reveals hemispheric differences in wave speed and attenuation in the outermost 100 km of the inner core (5, 7). Monnereau *et al.* propose an innovative explanation for this structure. Seismic anisotropy has also been reported for waves that travel deeper into the inner core. Advances in the analysis of normal mode observations by Deuss *et al.* provide new tools for probing the structure of the inner core.

Department of Earth and Planetary Science, University of California, Berkeley, CA 94720, USA. E-mail: bbuffett@berkeley.edu

anisotropy. Circulating flow due to thermal convection is one possibility (11). Interestingly, the observed seismic anisotropy also exhibits hemispherical variations. The strongest anisotropy is observed in the Western Hemisphere, which coincides roughly with the region where Monnereau *et al.* require melting. Lateral motion transports any existing crystal texture from west to east, where little anisotropy is observed. The absence of anisotropy in the east requires some mechanism to erase the crystal texture that originates in the west. There are several ways to accomplish this task (12), but a resolution requires further investigation.

One of the impediments to progress is a lack of observations. The inner core represents only 0.7% of Earth's volume, and the sampling provided by available seismic wave propagation paths is far from complete. Observations of normal modes (low-frequency oscillations of Earth as a whole) provide a complementary source of information, because the oscillation frequencies represent spatial averages of the elastic properties and density of Earth, including the inner core. However, conventional analysis of normal-mode observations cannot recover hemispherical structure because of

the nature of the averaging in this approach. These limitations can be overcome by accounting for the interaction of oscillations with nearby frequencies.

Deuss *et al.* now show that the additional work is worth the effort. A key step in their study lies in the choice of normal modes. The authors consider pairs of modes that have strong sensitivity to the inner core. One mode contributes to motion at the surface, whereas the other is typically confined to the inner core and would not be directly observed at the surface. In the presence of hemispherical inner core structure, these two modes interfere, perturbing the frequency of the first mode. By measuring these perturbations, the authors retrieve the hemispherical structure of the inner core. The results provide evidence for strong anisotropy in the west and relatively little anisotropy in the east. In fact, the region of strong anisotropy appears to be confined in longitude to a region that is narrower than an entire hemisphere. The origin of this feature is not known, but better observations, like those reported by Deuss *et al.*, are crucial to making progress.

Earth's solid inner core is an enigma. Expectations of a simple radial structure

have been replaced by hemispherical variations and other surprising complications. These features probably reflect events and processes that have occurred over geological time. Our ability to read the geological history of the core will ultimately depend on better data and new ideas about the structure of this tiny iron-rich sphere at Earth's center.

References

1. S. Labrosse, *Earth Planet. Sci. Lett.* **190**, 111 (2001).
2. X. L. Sun, X. D. Song, *Phys. Earth Planet. Inter.* **167**, 53 (2008).
3. M. Ishii, A. M. Dziewonski, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14026 (2002).
4. S. Tanaka, H. Hamaguchi, *J. Geophys. Res.* **102**, 2925 (1997).
5. M. Monnereau, M. Calvet, L. Margerin, A. Souriau, *Science* **328**, 1014 (2010); published online 15 April 2010 (10.1126/science.1186212).
6. A. Deuss, J. C. E. Irving, J. H. Woodhouse, *Science* **328**, 1018 (2010); published online 15 April 2010 (10.1126/science.1188596).
7. F. Niu, L. Wen, *Nature* **410**, 1081 (2001).
8. A. Cao, B. Romanowicz, *Earth Planet. Sci. Lett.* **228**, 243 (2004).
9. A. Morelli, A. M. Dziewonski, J. H. Woodhouse, *Geophys. Res. Lett.* **13**, 1545 (1986).
10. J. H. Woodhouse, D. Giardini, X.-D. Li, *Geophys. Res. Lett.* **13**, 1549 (1986).
11. B. A. Buffett, *Geophys. J. Int.* **179**, 711 (2009).
12. J. Wheeler, *Geophys. J. Int.* **178**, 1723 (2009).

10.1126/science.1190506

CELL SIGNALING

Signaling Through Cooperation

Emmanuel D. Levy,¹ Christian R. Landry,² Stephen W. Michnick¹

Living cells are complex systems that are constantly making decisions in response to internal or external signals. Among the most notable carriers of information are protein kinases and phosphatases, enzymes that receive inputs from cell surface or internal receptors and determine what actions should be taken in response, by phosphorylating or dephosphorylating substrates. How are these enzymes organized in the cell to capture and relay information in coordinated responses to signals? On page 1043 of this issue, Breitkreutz *et al.* (1) provide a key clue to this puzzle, describing how protein kinases and phosphatases in budding yeast are associated with other proteins and, most notably, with each other.

The structure of the kinase and phosphatase interaction network revealed by Breitkreutz *et al.* does not have the command and control organization suggestive of textbook-depicted linear cascades. Indeed, the authors observed about ~30% more interactions among kinases than would be expected by chance, suggesting extensive cross-talk between signaling pathways. This observation is also consistent with several recent findings—for example, kinases are more highly phosphorylated compared to other proteins, implying that kinases frequently target other kinases (2). Further, specific inhibition of a kinase results in extensive depletion (as expected) but also enrichment of phosphorylation of many proteins, suggesting substantial functional dependencies among kinases and phosphatases (3). Finally, kinases and phosphatases have more genetic interactions with each other than with other proteins, also pointing to substantial collaboration

Protein kinases and phosphatases may form a collaborative network of interactions to mediate cellular responses.

in the cell decision-making apparatus (4).

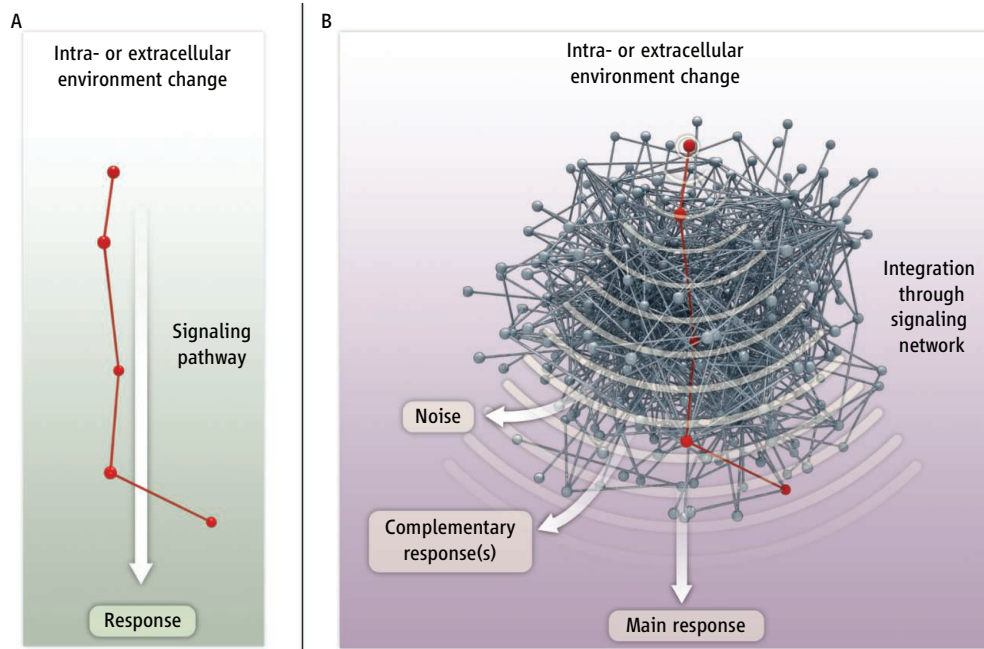
Conceptually, the kinase-phosphatase interaction network described by Breitkreutz *et al.* suggests a distributed organization of information flow. Such a structure might confer robustness to the system in that contributions to decisions are distributed across many proteins. Observations from genetic experiments are in line with this view, whereby relative contributions of proteins to cellular processes follow a continuum. Surely canonical components usually have the largest contribution, but many other proteins often exert a large influence (5) (see the figure). Interestingly, the kinase-phosphatase interaction network also resembles recent descriptions of transcriptional regulatory networks, seen as a collaborative layer rich in interactions among transcription factors (6, 7). Such a collaborative layer can be pictured as a table around which decision-makers debate a question and respond collectively to information put to them, akin to

¹Département de Biochimie, Université de Montréal, Montréal, Québec, Canada H3T 1J4. ²Université Laval, Département de Biologie, PROTEO and Institute for Integrative and Systems Biology, Québec, Québec, Canada. E-mail: stephen.michnick@umontreal.ca

a “democratic” network (6). The similarities between the kinase-phosphatase interaction and transcriptional regulatory networks could suggest evolutionary principles guiding the assembly of networks that organize information flow in cells. These networks have evolved from simpler ones and, to use the terms of the French biologist Jacques Monod, there might have been a great deal

phosphosites detected in phosphoproteomic screens, and hence of kinase-substrate interactions, that have no or little functional relevance. Compensation of phosphorylated sites (e.g., loss of one phosphosite but gain of another nearby) could help explain their limited evolutionary conservation (3), but the extent to which this mechanism occurs remains controversial (14).

Although the authors used standard affinity purification of “bait” proteins followed by mass spectrometry, they introduced important biochemical and computational twists. In particular, minimal washes of captured protein complexes were conducted to increase the detection sensitivity of unstable associations. In combination, they used a computational data-filtering scheme that



Network organization of cellular signaling. The kinase-phosphatase interaction network (B) revealed by Breitkreutz *et al.* contrasts with linear signaling cascades classically depicted in textbooks (A). The high degree of connections among kinases and phosphatases suggests a “board of directors” type of organization, whereby many proteins outside of the canonical pathway (in red) can influence its output. Such cooperative organization might allow the network to integrate several inputs and output complex responses. A certain degree of noise is expected to emanate from such a network.

of “necessity” at play that shapes their structure, but a lot of “chance” as well, the relative contributions of which have yet to be quantified (8, 9).

A side effect of the kinase-phosphatase interaction network collaborative structure is that it creates the conditions for indiscriminate chatter within and outside of the network, as seen for transcription factor–DNA binding networks (10). This is especially true given that substrate specificity is particularly degenerate among kinases and phosphatases (8, 11). Importantly, this could result in noisy interactions and phosphorylation events, even if recognition of substrates is augmented by auxiliary subunits or binding domains (12–14). Supporting this notion is the fact that phosphorylated sites (or “phosphosites”) of known function are more conserved than phosphosites of unknown function (8, 14). This leads to an estimated 50% or more of

The functional importance of phosphosites and kinase-phosphatase interactions could be better assessed on the basis of stoichiometry of phosphorylation and of interactions, and as importantly, on the basis of their conservation. By analogy, transcription factors appear to discriminate between nonspecific versus specific functional binding sites on the basis of their affinities; those sites with the highest affinity are likely those that are functional (15, 16). Similarly, the most abundant phosphosite on a given peptide is frequently the most conserved phosphosite on that peptide (13). Data on absolute phosphorylation abundance and kinase-phosphatase interaction intensity are not yet available, but may reveal a trend analogous to that seen for transcription factors. In this respect, the study by Breitkreutz *et al.* is an advance toward considering relative strengths and specificities of interactions.

enabled the differentiation of specific versus indiscriminate binding proteins on the basis of spectral counts. Such an approach might better reflect interactions that occur in the interior of cells, in that it relies more on the relative probabilities of interactions than on binary detection information under artificially stringent conditions. In parallel to such novel data acquisition and filtering approaches, careful analysis of how and at what rates phosphosites and kinase-phosphatase interactions have evolved should prove an efficient way to distinguish true functional signal from evolutionary noise in signaling networks.

Further progress in understanding signal transduction will require exploring the dynamic structure of kinase-phosphatase networks on both immediate and evolutionary time scales. This will require time-dependent experimental and computational reconstructions of local signaling networks (5) and a better understanding of the role of chance and necessity in shaping these networks.

References and Notes

1. A. Breitkreutz *et al.*, *Science* **328**, 1043 (2010).
2. A. Chi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 2193 (2007).
3. L. J. Holt *et al.*, *Science* **325**, 1682 (2009).
4. D. Fiedler *et al.*, *Cell* **136**, 952 (2009).
5. A. Friedman, N. Perrimon, *Cell* **128**, 225 (2007).
6. N. Bhardwaj, K. K. Yan, M. B. Gerstein, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 6841 (2010).
7. R. Jothi *et al.*, *Mol. Syst. Biol.* **5**, 294 (2009).
8. E. D. Levy, C. R. Landry, S. W. Michnick, *Sci. Signal.* **2**, pe11 (2009).
9. M. Lynch, *Proc. Natl. Acad. Sci. U.S.A.* **104** (suppl. 1), 8597 (2007).
10. G. Euskirchen, M. Snyder, *Nat. Genet.* **36**, 325 (2004).
11. J. Mok *et al.*, *Sci. Signal.* **3**, ra12 (2010).
12. J. A. Ubersax, J. E. Ferrell Jr., *Nat. Rev. Mol. Cell Biol.* **8**, 530 (2007).
13. C. R. Landry, E. D. Levy, S. W. Michnick, *Trends Genet.* **25**, 193 (2009).
14. A. N. Nguyen Ba, A. M. Moses, *Mol. Biol. Evol.* (10.1093/molbev/msq090) (2010).
15. S. Lin, A. D. Riggs, *Cell* **4**, 107 (1975).
16. S. MacArthur *et al.*, *Genome Biol.* **10**, R80 (2009).
17. E.D.L. and C.R.L. contributed equally to this work.

10.1126/science.1190993

CHEMISTRY

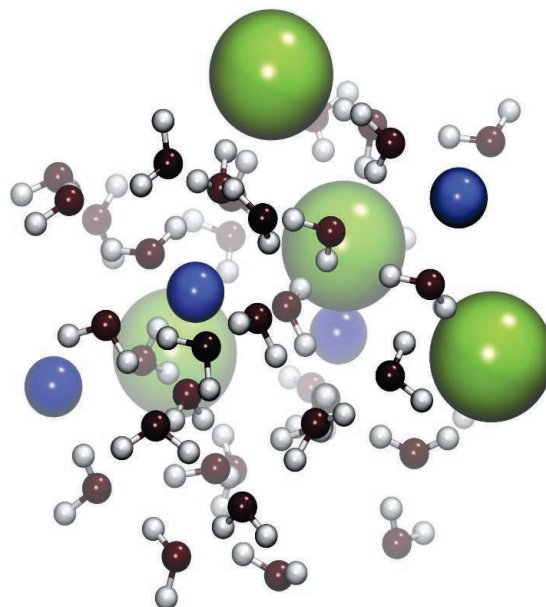
Following the Motions of Water Molecules in Aqueous Solutions

James L. Skinner

Water molecules in an aqueous solution constantly translate and rotate in an intricate and beautiful dance that underlies essential processes in areas ranging from biology to atmospheric chemistry. Recent experimental advances are letting us see some steps of this dance for water molecules interacting with simple molecules and ions. These studies are now possible because of the invention of infrared lasers that fire pulses on ultrashort time scales. In this issue, two examples at the forefront of this new field, involving the dynamics of water around anions and cations, are reported by Ji *et al.* on page 1003 (1) and Tielrooij *et al.* on page 1006 (2).

The isolated water molecule is very well understood from experiments and quantum-mechanical calculations. However, when a large number of water molecules form a liquid, our understanding of its structure and dynamics is more limited (3). Indeed, liquid water has a number of unusual properties (such as its high boiling point and density maximum at 4°C), which are thought to arise from intermolecular hydrogen bonds. It is especially important to understand the properties of water around ionic or hydrophobic solutes, which are also determined by hydrogen bonds (see the figure).

The rotational and translational motion of water molecules in solution can control the rates of important chemical and biochemical processes. These include chemical reactions on the surface of aqueous aerosol particles in the atmosphere and electron-transfer reactions critical for respiration and photosynthesis. Most of the experiments that have been used to measure the molecular dynamics of water involve the absorption or scattering of light with different wavelengths: radio waves in nuclear magnetic resonance studies, microwaves in dielectric relaxation, or infrared light in vibrational spectroscopy. Theoretical studies rely mainly on computer simulations in which the interactions among a collection of molecules are modeled with a potential energy function. The equations of



motion are integrated numerically, in effect creating a molecular movie.

The advent of new experimental and computational techniques is providing exciting information about water dynamics (4–7). The latest experiments use “ultrashort” infrared pulses with durations on the order of 50×10^{-15} s, or 50 fs. Such short pulses are necessary to resolve the elementary dance steps mentioned above. In the case of water, the infrared frequency is typically tuned to excite stretching vibrations of the OH bonds. The vibrational frequency of the OH group shifts when the H atom changes its hydrogen-bond environment. Experiments can measure these time-dependent fluctuations in the vibrational frequency, which is a process called “spectral diffusion.” The rotational dynamics of the OH bond can also be measured by using polarized light. Recent computational advances include new ways to analyze molecular trajectories from computer simulation (8, 9) and new ways to calculate vibrational spectra (10). Experiments and simulations for pure water have led to a detailed picture of “librational” motion (rotational motion that is hindered by hydrogen bonds) at very short times (less than 100 fs) (11), followed by rotational jumps and hydrogen bond making and breaking at

Ultrafast spectroscopy is revealing details of the rotational dynamics of water molecules in salt solutions.

Aqueous solvation. A schematic picture of water molecules interacting with cations (blue) and anions (green). Experimental studies by Ji *et al.* and Tielrooij *et al.* are revealing details of how water molecules rotate when ions are present and add to an emerging picture of water dynamics.

longer times (on the order of 2000 fs) (8).

In their study of an aqueous salt solution, sodium perchlorate (NaClO_4), Ji *et al.* exploited the distinct vibrational spectrum of water molecules hydrogen-bonded to other water molecules versus that of molecules hydrogen-bonded to perchlorate anions. In their polarization-selective “two-dimensional”

infrared experiments, an initial vibrational frequency is correlated with the frequency at a later time, which changes as the network of hydrogen bonds evolves. Ji *et al.* measured both spectral diffusion and rotational dynamics to create a detailed picture of the hydrogen-bond exchange mechanism. They showed that it occurs by means of large, very fast angular jumps, in agreement with a recently proposed model (9).

Tielrooij *et al.* studied a series of different ionic solutions with two techniques. Rotational dynamics were measured with polarization-resolved anisotropy decay, while low-frequency (terahertz) spectroscopy monitored intermolecular vibrations. For relatively dilute solutions, the effect of monovalent ions in water is very short ranged—only the water molecules next to the ion, that is, in its first solvation shell, are perturbed by the ion (6, 12, 13). Tielrooij *et al.* found that for some solutions (particularly MgSO_4 , which has divalent ions), the ionic perturbations extend for several solvation shells. Thus, the effects of the cations and anions can be nonadditive, which causes the water reorientation to be slower and involve more molecules.

These and related studies herald a new era in our understanding of solvation dynamics in aqueous solutions. We can now go on

Theoretical Chemistry Institute and Department of Chemistry, University of Wisconsin, Madison, WI 53706, USA.
E-mail: skinner@chem.wisc.edu

to study solvation of important small molecules (for example, the protein denaturant urea), and biomolecules such as peptides, proteins, nucleic acids, and membrane lipids, using similar techniques. The use of terahertz spectroscopy is particularly promising because it provides direct measurements of the low-frequency translational and rotational motions that are occurring in thermal equilibrium at room temperature. Experiments, coupled with new theoretical and computational techniques, will surely

shed new light on the critical problem of water dynamics around biomolecules and its effect on biomolecular function.

References and Notes

1. M. Ji, M. Odelius, K. J. Gaffney, *Science* **328**, 1003 (2010).
2. K. J. Tielrooij, N. Garcia-Araez, M. Bonn, H. J. Bakker, *Science* **328**, 1006 (2010).
3. P. Ball, *Nature* **452**, 291 (2008).
4. C. J. Fecko, J. D. Eaves, J. J. Loparo, A. Tokmakoff, P. L. Geissler, *Science* **301**, 1698 (2003).
5. J. B. Asbury *et al.*, *J. Phys. Chem. A* **108**, 1107 (2004).
6. H. J. Bakker, *Chem. Rev.* **108**, 1456 (2008).
7. H. J. Bakker, J. L. Skinner, *Chem. Rev.* **110**, 1498 (2010).
8. D. Laage, J. T. Hynes, *Science* **311**, 832 (2006).
9. D. Laage, J. T. Hynes, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 11167 (2007).
10. J. L. Skinner, B. M. Auer, Y.-S. Lin, *Adv. Chem. Phys.* **142**, 59 (2009).
11. D. E. Moilanen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 5295 (2008).
12. Y.-S. Lin, B. M. Auer, J. L. Skinner, *J. Chem. Phys.* **131**, 144511 (2009).
13. M. D. Fayer *et al.*, *Acc. Chem. Res.* **42**, 1210 (2009).
14. This research is supported by NSF (CHE-0750307) and the Department of Energy (DE-FG02-09ER16110).

10.1126/science.1190093

MATERIALS SCIENCE

Weight Loss with Magnesium Alloys

Tresa M. Pollock

The compelling need for lightweight, energy-efficient, environmentally benign engineering systems is driving the development of a wide range of structural and functional materials for energy generation, energy storage, propulsion, and transportation. These challenges motivate wider spread use of magnesium—the eighth most common element in the earth's crust and also extractable from seawater. In addition, the ease of recycling, compared with polymers, makes magnesium alloys environmentally attractive. Importantly, with a density of 1.74 g/cm³—about 30% less than aluminum, one-quarter that of steel, and nearly the same as many polymers—magnesium is attractive for lightweight structural systems and, most notably, automotive systems. A typical car weighing 1525 kg currently contains about 975 kg of steel, 127 kg of Al, 114 kg of polymeric materials, and 5 to 6 kg of magnesium (1). It is estimated that 22.5 kg of mass reduction would improve fuel efficiency by around 1%; thus, automotive manufacturers worldwide have goals to increase the Mg content of automobiles to between 45 and 160 kg (1, 2).

Among the widely available metallic elements that form the basis of engineering materials, magnesium is the most complex from the point of view of mechanical, chemical, and physical properties. Thus, its usage has been fairly limited (3). Interestingly, the current driving force for expanding use of Mg-based alloys occurs in an environment where the science of materials design and

processing can now address its complexities and offer up new possibilities.

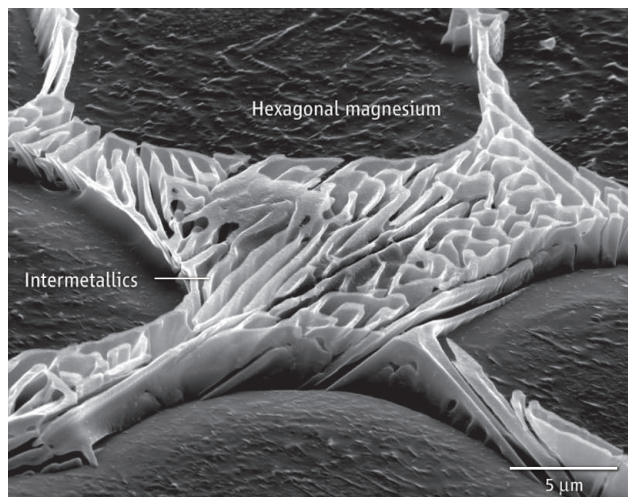
Load-bearing materials are often shaped by mechanical working processes such as rolling or extrusion that require high levels of stretching, that is, high ductility. This requires five independent modes of deformation at the atomic scale, accomplished by motion of dislocations. Unlike most engineering materials that have highly symmetric cubic crystal structures, Mg has a hexagonal structure with only two independent “easy” modes of deformation. As a result, failure may occur before activation of other required deformation modes such as dislocation glide, twinning, or grain boundary sliding. The emerging consensus is that deformation modes are more easily activated in

New design and processing approaches for magnesium-based alloys are offering a variety of opportunities in lightweight and energy-efficient applications.

materials with micro- or nanoscale grains, resulting in high ductility (4). Numerical simulations of the straining processes at the grain scale give further insights on the conditions under which large plastic strains can be achieved (5). Several fine-grain processing approaches are evolving to respond to this opportunity (6–13).

Ultimately, in a comprehensive design process, it is desirable to jointly optimize processing, structure, and composition. It is rare that a metallic material has its most favorable properties in pure elemental form; most engineering alloys are multicomponent, with a mixture of up to 5 to 10 elements. The higher-order elemental additions improve properties, typically by formation of additional beneficial phases within the

material, each possessing a crystal structure distinct from the base element. Controlling the formation of these “extra” phases is extremely important for Mg alloys that are cast into their final shape. Rare earth elements such as yttrium, lanthanum, cerium, and neodymium can improve the structure and properties of cast Mg alloys but substantially increase alloy cost. A recently discovered intermetallic C36 Laves-type phase forms during solidification as a result of additions of Al and Ca to magnesium (see the figure) (14). The unusual



Getting in the mix. Structure of an as-solidified Mg-Al-Ca base alloy showing hexagonal Mg phase (imaged dark) with plate-shaped C36 Laves Mg₂(Al,Ca) phase (imaged bright) that precipitates during cooling from above the melting point to 514°C.

Materials Department, University of California Santa Barbara, Santa Barbara, CA 93106–5050. E-mail: pollock@engineering.ucsb.edu

combination of mechanical and physical properties resulting from the low-cost Ca additions make the Mg-Al-Ca alloys promising for a variety of lightweight, high-temperature automotive powertrain components produced by die casting (15).

The optimization of multicomponent materials has largely been accomplished empirically, with the appreciable cost, time, and effort presenting a major barrier to use of new materials (16). However, breakthroughs in the understanding of the basic thermodynamics and kinetics of multicomponent metallic systems have led to more rapid design of new alloys with favorable compositions and mixtures of phases (17). A new development in this design approach for Mg alloys is the direct input of first principles calculations to the design process. Ab initio codes have been used to predict lattice parameters, elastic properties, enthalpies of formation, and the relative stabilities of the complex series of Laves phases that exist in quaternary and higher-order Mg-Al-Ca-X systems (18, 19). This information can be input to newly developed thermodynamic databases (20) and can then be used to select compositions with desirable combinations of phases and corresponding thermal processing paths. The availability of high-fidelity modeling tools permits a greater compositional space to be explored, increasing the likelihood that new, optimal solutions are identified.

Other unique properties of magnesium-based systems motivate new application realms. The electrochemical properties of Mg make it an interesting material for rechargeable batteries (21), including all-liquid batteries for storage of solar energy. Corrosion, another electrochemical process, can be controlled in a manner such that Mg-based implants with controlled rates of biodegradation are feasible (22). Good electromagnetic and radio frequency shielding along with high specific strength make Mg attractive for cell phones, computer cases, cameras, and other communication devices (2). Unlike aluminum, magnesium alloys can be cast to sections as thin as 0.5 mm, providing further incentive for incorporation of Mg into lightweight mobile electronics.

Will Mg alloys be considered with equal confidence in comparison with polymers and other metallic systems as solutions for energy and lightweight-materials challenges? The answer is likely "yes," particularly if the rapidly evolving fundamental understanding of the properties of this unique class of materials is captured in models that can be integrated and used for

the design of new materials and for prediction of their performance.

References and Notes

- G. S. Cole, in *Magnesium Technology 2007*, R. S. Beals et al., Eds. (Minerals, Metals, and Materials Society, Warrendale, PA, 2007), pp. 35–40.
- E. Aghion, B. Bronfin, *Mater. Sci. Forum* **350–351**, 19 (2000).
- S. Mathaudhu, E. Nyberg, in *Magnesium Technology 2010*, S. R. Agnew et al., Eds. (Minerals, Metals, and Materials Society, Warrendale, PA, 2010), pp. 27–33.
- J. Koike, *Metall. Mater. Trans.* **36**, 1689 (2005).
- C. J. Neil, S. R. Agnew, *Int. J. Plast.* **25**, 379 (2009).
- R. Decker, S. Kulkarni, J. Huang, S. Lebeau, in *Magnesium Technology 2009*, H. J. Kaplan, Ed. (Minerals, Metals, and Materials Society, Warrendale, PA, 2009), pp. 357–361.
- J. Swiostek, J. Goken, D. Letzig, K. U. Kainer, *Mater. Sci. Eng. A* **424**, 223 (2006).
- K. Matsubara, Y. Miyahara, Z. Horita, T. G. Langdon, *Acta Mater.* **51**, 3073 (2003).
- D. St. John, M. Qian, M. A. Easton, P. Cao, Z. Hildebrand, *Metall. Mater. Trans.* **36**, 1669 (2005).
- H.-S. Di et al., *Mater. Sci. Forum* **488–489**, 615 (2005).
- S. M. Zhang, Z. Fan, Z. Zhen, *Mater. Sci. Technol.* **22**, 1489 (2006).
- W.-J. Kim, G. Lee, J. Lee, *Adv. Eng. Mater.* **11**, 525 (2009).
- G. Cao et al., *Mater. Sci. Eng. A* **494**, 127 (2008).
- A. Suzuki, N. D. Saddock, J. W. Jones, T. M. Pollock, *Acta Mater.* **53**, 2823 (2005).
- B. R. Powell, A. A. Luo, V. Rezhets, J. J. Bommarito, B. L. Tiwari, "Development of Creep-Resistant Magnesium Alloys for Powertrain Applications: Part 1 of 2," SAE Paper 2001-01-0422, SAE, Pittsburgh, PA (2001).
- National Research Council, *Integrated Computational, Materials Engineering* (National Academies Press, Washington, DC, 2008).
- G. B. Olson, *Science* **288**, 993 (2000).
- Y. Zhong, J. Liu, R. Witt, Y. Sohn, Z. Liu, *Scr. Mater.* **55**, 573 (2006).
- W.-Y. Yu et al., *Solid State Sci.* **11**, 1400 (2009).
- A. Janz et al., *Acta Mater.* **57**, 682 (2009).
- E. Levi, Y. Gofer, D. Aurbach, *Chem. Mater.* **22**, 860 (2010).
- N. Hort et al., *Acta Biomater.* **6**, 1714 (2010).
- I acknowledge useful discussions with J. E. Allison, R. D. Decker, J. W. Jones, S. N. Mathaudhu, and B. R. Powell. I gratefully acknowledge N. D. Saddock for input on the figure. I also acknowledge the National Science Foundation for support of research on Mg alloys.

10.1126/science.1182848

BIOCHEMISTRY

Stochastic Emergence of Groupthink

Arthur Prindle and Jeff Hasty

Oscillations in the synthesis and release of a chemical signal synchronize the behavioral response of a cell population.

Oscillations are found at nearly every level of biology. From the dynamic instability of cytoskeletal elements in an individual cell to the circadian rhythms that regulate a multitude of operations at the organismal level, it is clear that periodicity is an essential characteristic of living systems. On page 1021 of this issue, Gregor et al. (1) describe how cell aggregation and development of the amoeba *Dictyostelium discoideum* is guided by emergent rhythmic behavior arising from the stochastic pulsing of individual cells with a chemical cue. By combining experimental and computational approaches, the authors present the exciting story of the dynamical onset of collective behavior in this organism. The findings raise the question of whether biology uses oscillations to solve problems typically assumed to have static or unidirectional solutions.

D. discoideum is a "social" amoeba in

that it transitions from a collection of unicellular organisms to a multicellular slug and finally to a fruiting body during its life cycle (see the figure). Cell aggregation and the commitment to form a fruiting body are regulated through cell-to-cell signaling by the small molecule cyclic adenosine monophosphate (cAMP). With the rhythmic synthesis and release of the chemoattractant cAMP, *D. discoideum* coordinates its collective behavior by aggregating to the region of highest cell density and initiating the formation of a fruiting body only when sufficient accumulation of cAMP is achieved. Before this threshold is met, cells act independently and do not aggregate. This marked synchronization, called dynamical quorum sensing, occurs when individual oscillators are coupled through a common field, often comprising extracellular signaling molecules in biological settings (2, 3). This highly dynamic process ensures that the cell population behaves efficiently and differentiates only when conditions are desirable. Whether this synchronized behavior is initiated and gov-

Department of Biology and Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA. E-mail: hasty@bioeng.ucsd.edu

erned by the activities of a few specialized cells or is an emergent collective phenomenon has been an open question.

In the study by Gregor *et al.*, individual cells and populations were observed in a perfusion chamber to study their dynamics in a controlled environment. A fluorescence resonance energy transfer (FRET)-based sensor monitored the amount of cytosolic cAMP directly. The authors probed the behavior of the system under varying amounts of extracellular cAMP, measuring the oscillatory frequency of intracellular cAMP production by cells each time. It is this precise variation of parameters that permits the analysis of a complex system such as the *D. discoideum* collective. Gregor *et al.* found that intracellular oscillations in cAMP



Emergent phenomena. The social amoeba *D. discoideum* is a single-cell organism that, when collected together at sufficiently high density, exhibits emergence in its cellular aggregation (left). A common example of emergent phenomena in nature is the snowflake, where the macroscopic shape is dictated by the molecular geometry of the water molecule (right).



concentration could occur even when the environmental concentration of cAMP was held constant and far above the saturation level of the cAMP receptors, demonstrating that the origin of oscillations is internal to the cell and does not require extracellular cAMP to be periodically cleared. By driving the internal cAMP oscillation system with periodic fluctuations in extracellular cAMP, the authors identified a single dominant frequency of internal cAMP oscillation above and below which the population-level oscillation diminished over time. This natural frequency is determined by the intracellular oscillation machinery and provides an internal timekeeping mechanism. Future biochemical studies on the origin of oscillations may use this frequency as a starting point in thinking about the types of biological processes that match the time scale.

In their computational modeling, Gregor *et al.* used a phase equation to describe the oscillatory state of a single cell. Equations

like this are sometimes called “theta models” because the state of the system is given by a periodic variable θ , often represented as an angle on a circle. Such models naturally lend themselves to cyclical processes and have been used to describe oscillations in a variety of engineering and biological contexts such as the synchronization of flashing in fireflies. Upon coupling many of these single-cell models together computationally via the common field (cAMP), the behavior of the single-cell models began to synchronize and dynamical quorum sensing emerged. The population of single-cell models switched to a synchronized oscillatory state when a critical cell density was reached, as observed experimentally. They reproduced the experimentally measured relationship between cell

density and frequency of collective cAMP oscillation only when the stochastic pulsing of individual cells in response to low, subthreshold concentrations of extracellular cAMP was included in the model. This is perhaps the most intriguing result because it confirms that random pulses of cAMP production at subthreshold levels of extracellular cAMP can affect the collective interactions of individual cells and initiate rhythmic synthesis of cAMP across a population. That is, organized group behavior originates from random individual behavior. When the population is at very low density, individual cells are “excited” by external cAMP infrequently and randomly without regard for their neighboring cells. With each pulse of excitation by extracellular cAMP, cAMP is synthesized intracellularly and secreted, increasing the local probability of excitation and creating chain reactions that produce patches of synchronized cAMP synthesis. These synchronized patches continue to excite nearby

cells, producing a snowball effect. Multiple oscillatory patches can arise in this way, and a competition between them ensues. Those that produce more cAMP can “pulse” (synthesize cAMP) at higher frequencies, entraining and attracting patches that pulse at lower frequencies. As each new patch is conquered, the size and oscillatory frequency of the victorious patch increases. A single dominant frequency is ultimately reached, coordinating the behavior of the population.

In addition to providing a beautiful description of collective behavior in *D. discoideum*, the work of Gregor *et al.* offers new insights into the underlying mechanisms of control in living systems. It provides a detailed look at emergent biological phenomena—unpredictable, complex behaviors arising from a large number of relatively simple interactions. Although the mechanism of oscillation remains elusive, clues may be found in recent synthetic circuits that produced similar emergent behavior (4). Using simple gene circuits, emergent oscillatory behavior was achieved by a population of cells with a common extracellular signaling molecule, N-acyl homoserine lactone (AHL), which performs the dual roles of activating the genes necessary for intracellular oscillations of AHL and mediating the coupling between cells (4). It may be that cAMP acts similarly in *D. discoideum*.

Cellular aggregation and differentiation are behaviors critical to the survival of an organism, and yet the work of Gregor *et al.* demonstrates that random, decentralized processes initiate them in *D. discoideum*. It may seem surprising that biology would take this approach rather than leaving this decision in the hands of specialized pacemaker cells with more elaborate timekeeping and nutrient-sensing mechanisms. Is such stochasticity merely limited to lower organisms, or might it be an underlying property that guides all of biology? If so, how can this be reconciled with the homeostatic picture we observe macroscopically? Contending with these questions may reveal new fundamental properties of living cells and their methods of control.

References

1. T. Gregor *et al.*, *Science* **328**, 1021 (2010); published online 22 April 2010 (10.1126/science.1183415).
2. A. F. Taylor, M. R. Tinsley, F. Wang, Z. Huang, K. Showalter, *Science* **323**, 614 (2009).
3. S. De Monte, F. d'Ovidio, S. Danø, P. G. Sørensen, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 18377 (2007).
4. T. Danino, O. Mondragón-Palomino, L. Tsimring, J. Hasty, *Nature* **463**, 326 (2010).

10.1126/science.1190372

Primordial Gravitational Waves and Cosmology

Lawrence M. Krauss,^{1*} Scott Dodelson,^{2,3,4} Stephan Meyer^{3,4,5,6}

The observation of primordial gravitational waves could provide a new and unique window on the earliest moments in the history of the universe and on possible new physics at energies many orders of magnitude beyond those accessible at particle accelerators. Such waves might be detectable soon, in current or planned satellite experiments that will probe for characteristic imprints in the polarization of the cosmic microwave background, or later with direct space-based interferometers. A positive detection could provide definitive evidence for inflation in the early universe and would constrain new physics from the grand unification scale to the Planck scale.

Observations made over the past decade have led to a standard model of cosmology: A flat universe dominated by unknown forms of dark energy and dark matter. Although all available cosmological data are consistent with this model, the origin of neither the dark energy nor the dark matter is known. The resolution of these mysteries may require us to probe back to the earliest moments of the Big Bang expansion, but, before a period of about 380,000 years, the universe was both hot and opaque to electromagnetic radiation. Thus, to probe back to earlier times we need to search for other observables outside of the electromagnetic spectrum.

Gravitational waves, predicted in general relativity, interact very weakly with matter, and therefore the universe has been transparent to them since shortly after the Big Bang. As a result, they provide a potentially new probe of early universe cosmology, that is, if we can detect them. Here, we review the most likely early universe sources of a detectable gravitational wave background and also explore the most likely detection method, via a measurement of polarization in the cosmic microwave background (CMB) radiation-remnant thermal radiation from the Big Bang, which has propagated unimpeded to our detectors since the time that the universe first became transparent to electromagnetic radiation.

Primordial Gravitational Waves

Just as in electromagnetism moving charges can generate electromagnetic waves, in general relativity moving masses can generate gravitational waves.

Because of the weakness of gravity, astronomical amounts of matter must be moved around in order to generate waves on a scale that might actually be detectable. Direct detectors, such as the Laser Interferometer Gravitational-Wave Observatory (LIGO) detector (*1*), are being designed to measure the local space-time distortion generated by gravitational waves from astrophysical objects such as colliding neutron stars or black holes.

However, there is another cosmological source of gravitational waves that can arise from the dynamics of our expanding universe at very early times. Such a background will involve waves today whose wavelengths will extend all the way up to our present cosmological horizon (the distance out to which we can currently observe in principle) and that are likely to be well beyond the reach of any direct detectors for the foreseeable future.

Primordial gravitational waves also leave indirect signatures that might show up in CMB maps. Primordial mass and energy density fluctuations grow to produce cosmological structures

and also create observable CMB temperature anisotropies. Primordial gravitational waves, on the other hand, produce not only temperature anisotropies but also a distinctive signal that can be detected with very sensitive measurements of the polarization of the CMB. Although temperature anisotropies have been observed, current experiments have only been able to put upper limits on polarization anisotropies that might result from a gravitational wave background.

However, there are reasons to believe that such a background exists and should be observable in the next generation of CMB polarization experiments. The observed pattern of temperature anisotropies, combined with that of inhomogeneities in matter on large scales, and measurements of the total energy density in the universe are in striking agreement with the simplest predictions of so-called inflationary models (*2–5*).

Inflation suggests that, early in its history, the universe underwent a brief epoch of accelerated expansion during which small-scale fluctuations were stretched to superhorizon scales. The theory posits that quantum mechanical fluctuations in the fields that describe the matter content at early times seeded the structure in the universe, leading to two important signatures: (i) primordial anisotropies imprinted in the CMB at a level of a few parts in a hundred thousand (Fig. 1) and (ii) an inhomogeneous matter distribution in the form of a cosmic web, as traced for example by the location of galaxies in the universe (Fig. 2).

The inflationary paradigm is remarkably consistent with the detailed statistical features of both of these observed signatures, which can arise from scalar density perturbations generated during an early inflationary phase. But scalar density perturbations are not the only perturbations that are generated. Gravitational waves are also produced [see supporting online material (SOM) for details].

When some background field gets stuck in a metastable state, energy gets stored in space,

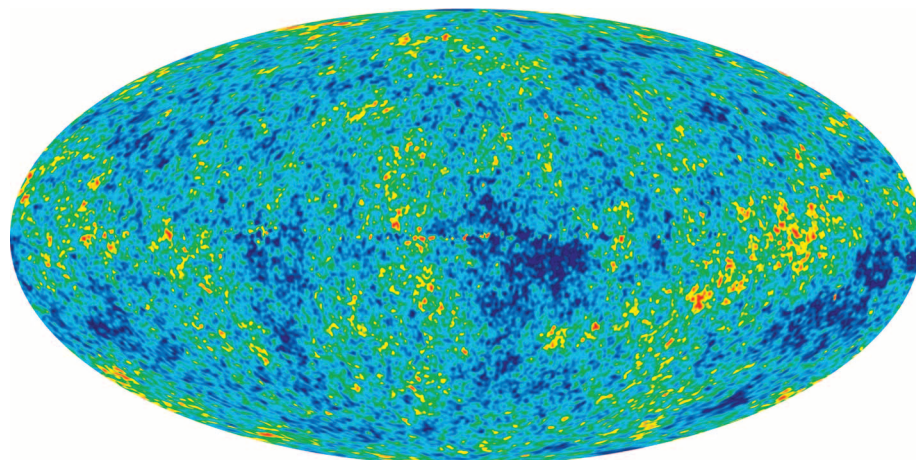


Fig. 1. Wilkinson Microwave Anisotropy Probe (WMAP) 7-year map of CMB temperature anisotropies across the sky, with galactic foregrounds removed. Color differences represent relative temperature anisotropies on the order of 10^{-5} of the uniform background temperature. [Credit: WMAP Science Team]

¹School of Earth and Space Exploration and Department of Physics, Arizona State University, Post Office Box 871404, Tempe, AZ 85287, USA. ²Center for Particle Astrophysics, Fermi National Laboratory, Post Office Box 500, Batavia, IL 60510, USA. ³Department of Astronomy and Astrophysics, University of Chicago, 5640 South Ellis Avenue, Chicago, IL 60637, USA. ⁴Kavli Institute of Cosmological Physics, University of Chicago, 5640 South Ellis Avenue, Chicago, IL 60637, USA. ⁵Department of Physics, University of Chicago, 5640 South Ellis Avenue, Chicago, IL 60637, USA. ⁶Enrico Fermi Institute, University of Chicago, 5640 South Ellis Avenue, Chicago, IL 60637, USA.

*To whom correspondence should be addressed. E-mail: krauss@asu.edu

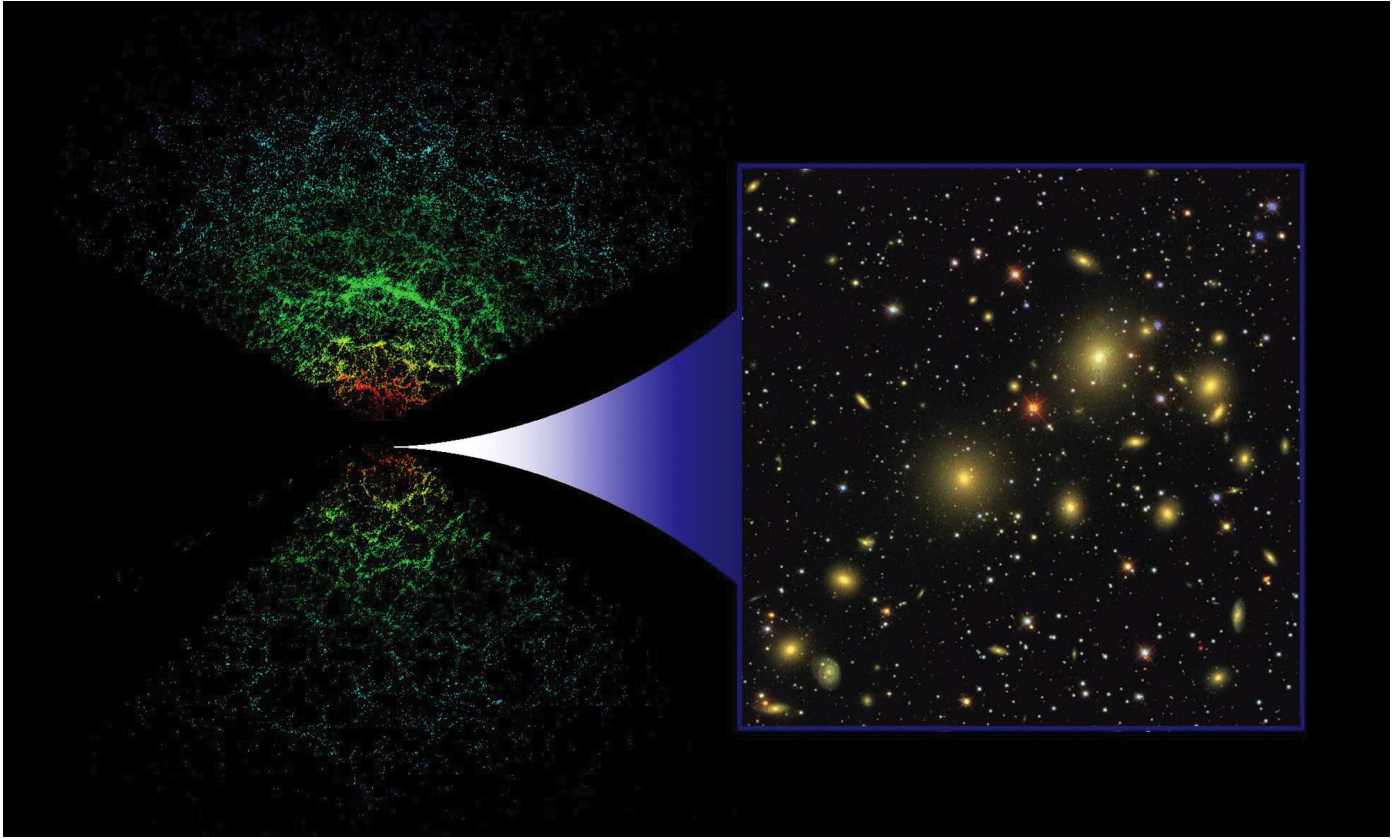


Fig. 2. A map of the observed distribution of galaxies on large scales (from the Sloan Digital Sky Survey), illustrating vast structures separated by large voids. The standard cosmological model, built on inflation, explains the origin of the WMAP image in Fig. 1 and how it evolved into the present-day distribution of large-scale structure.

acting like a cosmological constant and producing a negative pressure and a positive energy density. Solving Einstein's equations in such a background results in an accelerated expansion that quickly becomes exponential. The appropriate metric for such a universe is the de Sitter metric.

In a de Sitter background, all massless or light quantum fields will fluctuate with a magnitude that is proportional to the only dimensional parameter governing the expansion, the stored energy density, ρ , which by Einstein's equations is related to the square of the Hubble expansion parameter, H , at the time. This implies that fluctuating background gravitational waves, which in the quantum theory correspond to massless quantum fields, will also be generated. As inflation stretches distances, their wavelengths soon become much larger than the Hubble radius—they are driven outside the horizon—after which their amplitude remains fixed because no causal process can act over distances larger than the horizon.

After inflation ends, these modes return inside the horizon as a stochastic background of gravitational waves with a power that is proportional to H^2 (6–9). Clearly, the larger the energy scale at which inflation occurs, the larger will be the residual gravitational wave signal. This signal is thus determined solely by the energy scale at which inflation may have occurred.

In the context of the CMB as we shall describe, such modes can leave an imprint in the polarization of radiation. One can then compare the predicted polarization power spectrum to the power spectrum for direct observed temperature anisotropies in the CMB (SOM1).

The quantity r , defined as the ratio of these two power spectra (9), plays a definitive role in parameterizing the detectability of gravitational waves in CMB experiments. The next generation of detectors is being designed with the intention of reaching a sensitivity to $r \geq 0.01$. For the simplest models involving a single field, once r is measured, the energy density during inflation will also be determined; the two are related via $V^{1/4} = 1.06 \times 10^{16} \text{ GeV } (r/0.01)^{1/4}$. Current limits of $r < 0.3$ already have severely constrained or ruled out a variety of single-field inflationary potentials and give hope that increasing the experimental sensitivity to r could yield a positive nonzero signature for gravitational waves in the near future.

At the same time, there is an independent argument that suggests that one should be cautious before counting on such a possibility. Inflation requires an expansion factor of at least e^{60} in order to resolve all the cosmological puzzles that it was designed to resolve. One can ask what the total field excursion is during this period, and the larger the scale of inflation, the larger the

magnitude of the inflaton field at the end of inflation. One finds, in fact, again for single-field inflation (9) that $\Delta\phi/M_{\text{pl}} \geq 1.06 \times (r/0.01)^{1/2}$, where M_{pl} is the Planck mass. Field excursions larger than the Planck scale lead to territory where Planck-scale effects may be important, producing possibly large undetermined corrections to simple quantum field theoretic estimates. In fact, in many string theory models the value of the inflating field is associated with an excursion in an extra dimension, which is itself restricted to be Planck length in size so that it is impossible for the field to take values larger than the Planck scale, and therefore one would expect that $r < 0.01$. More recently, it has been realized that string theory might allow for cases where r can exceed 0.01 (10) so that distinguishing between small and large r would help define the symmetry structure of the theory. Thus $r = 0.01$ represents an important threshold.

Because inflation produces gravitational waves on all scales, one would hope that other probes of gravitational waves with smaller wavelengths and higher frequencies might be useful. But once inside the horizon, gravitational waves redshift like radiation. During matter domination, the contribution to the total energy density in such modes falls by a factor identical to that of photons, so that their contribution to the energy density today will be suppressed.

Lastly, inflation is not the only mechanism that might produce a stochastic gravitational wave background. For example, a phase transition in the early universe could result in a background whose spectrum inside the horizon is remarkably similar to that from inflation (11). Detailed calculations suggest that the signal from such a transition can be enhanced (12–14) so that even transitions somewhat below the scale of inflation could produce a comparable signal. Various different possible ways of distinguishing this signal have been suggested, including looking at the real space correlations of polarization (15), exploring the three-point correlation function for polarization (16), and comparing a possible CMB gravitational wave signal with a future signal in direct detectors (13).

What Can We Learn from CMB Polarization?

The greatest sensitivity to a primordial gravitational wave background comes from the detailed pattern of polarization in the CMB (17–19). Thomson scattering of an anisotropic radiation background off of free electrons before the time of electron-proton recombination (when the universe was 380,000 years old) produced a radiation field polarized at the 10% level. Because polarization is described at every position by an amplitude and an angle of orientation, the polarization field on the sky can be decomposed into two modes, a curl-free E mode and a divergence-less B mode (Fig. 3). The B mode pattern cannot be produced by scalar perturbations; thus, its detection would be a signature of primordial gravitational waves.

Although the E modes are predominantly generated from scalar perturbations, they are nonetheless important to those who study inflation: They have been detected, and the observed pattern of E modes is in perfect agreement with the expectations from inflation. Inflation predicts that the temporal phases of all perturbations were set early in the history of the universe. This primordial coherence leaves its mark on the anisotropy pattern (20). Because polarization is produced by Compton scattering of anisotropic radiation off electrons, the spectrum of temperature anisotropies is predicted to be closely related to the E mode spectrum. The E modes reach their maximum and minimum amplitudes at precisely the same times as do the higher moments (dipole and quadrupole) of the radiation field. In contrast, the temperature pattern that we observe is the monopole of the radiation field at

recombination. The continuity equation dictates that the monopole and dipole are out of phase with one another, so the E mode of polarization is 90° out of phase with the acoustic pattern in the temperature spectrum (Fig. 4). This phase lag has been observed even on scales larger than one degree, which were not in causal contact when the universe was 380,000 years old. The phases were set up at very early times, long before recombination, just as predicted by inflation.

The B modes are the next frontier. Although the amplitude of the signal is unknown, the shape of the spectrum is robustly predicted by theory (Fig. 4). To understand the shape, recall that the polarization signal is generated only during those times when electrons are not trapped in neutral hydrogen but are free to scatter off of radiation. However, if scattering is very efficient the resulting polarization signal will be very small. Imagine a free electron in the early universe. Scattering off of this electron will produce polarization only if the observed incoming radiation

and protons are in the process of recombination at $t = 380,000$ years old and (ii) much later, when electrons are liberated from neutral hydrogen but the universe is so dilute that scattering is rare. The signals generated at these two times are imprinted onto the characteristic scales associated with each: the horizons $d_H(z = 1100)$ and $d_H(z \approx 10)$. We observe these at angular scales equal to $\theta = d_H/d_A(z)$, where $d_A(z)$ is the angular diameter distance to redshift z . Hence, the predicted signal with bumps at angular scales of $\sim 2^\circ$ and $\sim 50^\circ$.

One complication that is also apparent in Fig. 4 is the effect of gravitational lensing (21, 22). Scalar perturbations produce only E modes at recombination, but the radiation from this last scattering surface travels through a clumpy universe before it reaches us. The ensuing gravitational lensing generates B modes from the primordial E field. These become important on smaller scales than those on which the B humps appear, but they limit our ability to detect the higher B harmonics. Although techniques have been proposed to clean this E leakage, the most promising approach appears to be to measure the two peaks at $\sim 2^\circ$ and $\sim 50^\circ$.

Experimental Efforts, Present and Future

The current generation of direct gravitational wave detectors, such as the LIGO detector (1), do not have sufficient sensitivity to probe for possible primordial gravitational waves. Direct detection will require more sensitive space-based interferometers, with a further improvement of at least three to four orders of magnitude in sensitivity at the very least required. Because of the technological challenges and cost, such detectors are likely to take at least one or two decades to develop, and even then the likelihood of achieving the necessary sensitivity is not yet clear.

Rapid progress in the measurement of CMB temperature and polarization fluctuation spectra may allow the detection of primordial gravitational waves within the decade (SOM2). The experiments are carried out from ground-based observatories, high-altitude balloons, and satellites in space. Satellites operate for years, making sensitive, all-sky maps that are not contaminated by atmospheric emission and that can therefore measure large angular-scale fluctuations. Satellite telescopes are by necessity small and therefore have limited resolution, angles above 0.2° . As with satellites, balloon-borne experiments are free of most of the interfering emission from the atmosphere and have the additional advantage of a relatively short development cycle. New, sensitive, high-pixel count focal planes currently give balloon experiments the highest instantaneous sensitivity, but flights are limited to a couple of weeks at the maximum. Ground-based experiments, on the other hand, can have large-diameter telescopes, making possible high angular resolution. The telescopes can operate for many years and have readily accessible instrumentation. These experiments have evolved from a first detection of temperature anisotropy in 1992 to the

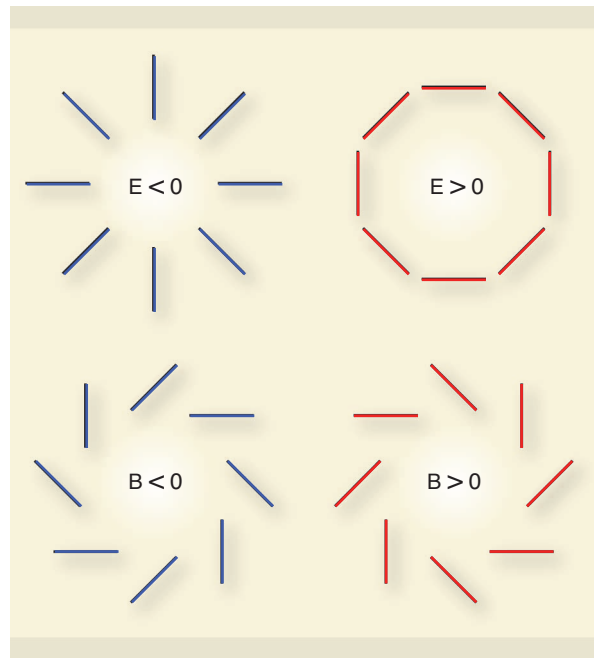


Fig. 3. Polarization can be decomposed into E and B modes. The former are radial or tangential with no preferred handedness, akin to an electric field. Like magnetic fields, B modes do have handedness; note that if reflected across a line going through the center the E patterns are unchanged, whereas the positive and negative B patterns get interchanged. This peculiar pattern can be produced only by gravitational waves, not by ordinary density perturbations.

field is anisotropic. If scattering is very efficient, the mean free path of photons will be very small, and the last scattering surface that the electron observes will be very nearby. Thus, all incoming radiation will share the same temperature, and there will be no anisotropy from which to generate polarization. Polarization then requires scattering but not rapid scattering. This set of requirements limits the epochs during which polarization is generated to two: (i) when electrons

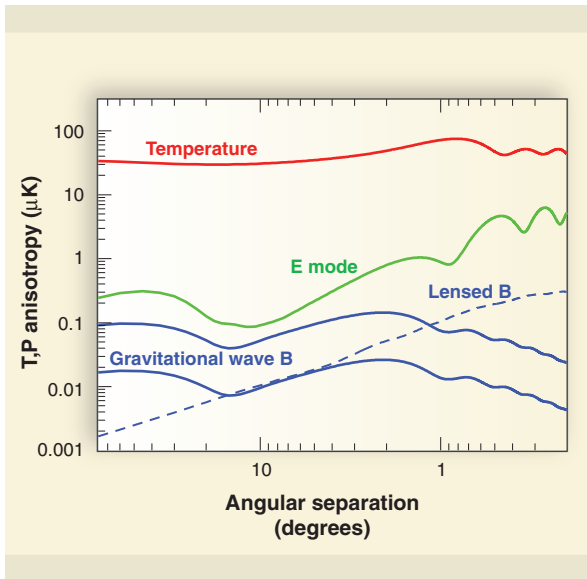


Fig. 4. Root mean square fluctuations in temperature (T) and polarization (P) of the CMB predicted by inflation. The temperature spectrum has been measured with exquisite precision by dozens of experiments, culminating in WMAP, whereas there have been half a dozen detections of *E* modes. There are currently only upper limits on the amplitude of *B* modes. The top *B* mode curve represents the current upper limit, $r = 0.3$, and the bottom curve represents the value $r = 0.01$.

detection of CMB polarization in 2002 to sensitive probes of *B* modes today, a gain in sensitivity of over 10^5 in just 17 years.

Three elements combine to specify the overall power of experiments to probe the CMB inflation signal. The first is sensitivity. The CMB polarization signals are exceedingly weak, a few millionths of a degree, but detectors must cope with thermal emission of the telescope and the environment. Only space instruments can be cold enough to avoid local emission. State-of-the-art detectors today are limited by the fundamental photon noise, and so the sensitivity of individual detectors cannot be improved much. However, large focal plane instruments with thousands of detectors in effect make many simultaneous measurements. Most of the improvement in instrument sensitivity over the past decade is the result of ever-increasing detector count and thus instrument complexity. Plans for ground-based and satellite instruments with tens of thousands of detectors are underway, and these will have the sensitivity needed to measure the two bumps in the *B* mode polarization in Fig. 4.

The second element controlling the power of experiments is their ability to differentiate CMB structure from structure in foreground emission, particularly diffuse emission from our own Galaxy. Unfortunately, we know very little about this emission and nearly nothing about its polarization properties apart from the fact that it is

partially polarized. The maximum polarization signal consistent with what we know already is subdominant to galactic emission in all but a few places in the sky in a limited range of frequency. A high-fidelity map of pure primordial *B* mode polarization over a substantial area on the sky requires the ability to discriminate and remove the galactic signal. Removal can be done by observing at multiple frequencies and noting that the galactic emission has a different spectrum or color than the CMB. Models of the foregrounds and the process of foreground removal leave reason for optimism about the prospects of making clean maps. However, knowledge of the real properties of the emission awaits the results of the currently operating Planck satellite, and we currently do not really know how well we can remove foregrounds from CMB maps.

The third experimental element is control of instrument systematic uncertainties. The *B* mode signal constitutes a particular pattern of polarization, but the sought-for strength is nine orders of magnitude weaker than the uniform glow of the CMB itself and three orders of magnitude weaker than the *E* mode polarization. A very subtle shift or rotation of a detector or a glint of radiation from out of the field could mimic a signal. The design of the instruments, the program of mapping and observation, and the experimental characterization and calibration will all need to be carried out with an unparalleled level of precision.

The increase of the raw sensitivity of CMB experiments has been rapid and is the key factor leading to more powerful measurements. Although many current experiments are close to being limited by previously unanticipated systematic effects, each generation has led to better instrument design and enhanced immunity to systematic error. This iterative method is permitting rapid progress on all three elements limiting power of the measurement.

The technology to build sufficiently sensitive experiments is in hand. Previous gains in sensitivity were the result of new detectors. Further improvement will come from increasing the number of detectors. Currently experiments have 1000. The next generation will have 10,000, and a proposed satellite has 50,000. The construction of focal planes of this scale and sensitivity will require the sustained support of detector design, development, and testing (23).

The Planck satellite (24), currently operating, will provide new data on polarization within the next 3 to 4 years and might even provide the first direct observation of *B* modes. Either way, the results from Planck will govern the ultimate design of the next generation of dedicated CMB polarization experiments in space. From design to construction to launch and operation could take the better part of a decade. As we enter the second decade of the 21st century, we are thus poised to enter a new realm of precision cosmology, one that could provide a dramatic new window on the early universe and the physical processes that governed its origin and evolution.

References and Notes

1. B. P. Abbott *et al.*, <http://arxiv.org/abs/0909.3583v3> (2009).
2. A. H. Guth, *Phys. Rev. D* **23**, 347 (1981).
3. A. D. Linde, *Phys. Lett. B* **108**, 389 (1982).
4. A. Albrecht, P. J. Steinhardt, *Phys. Rev. Lett.* **48**, 1220 (1982).
5. A. D. Linde, *Phys. Lett. B* **129**, 177 (1983).
6. V. A. Rubakov, M. V. Sazhin, A. V. Veryaskin, *Phys. Lett. B* **115**, 189 (1982).
7. A. A. Starobinsky, *J. Exp. Theor. Phys. Lett.* **30**, 682 (1979).
8. L. M. Krauss, M. White, *Phys. Rev. Lett.* **69**, 869 (1992).
9. D. Baumann *et al.*, <http://arxiv.org/abs/0811.3919> (2008).
10. E. Silverstein, A. Westphal, *Phys. Rev. D* **78**, 106003 (2008).
11. L. M. Krauss, *Phys. Lett. B* **284**, 229 (1992).
12. K. Jones-Smith, L. M. Krauss, H. Mathur, *Phys. Rev. Lett.* **100**, 131302 (2008).
13. L. M. Krauss, K. Jones-Smith, H. Mathur, J. Dent, <http://arxiv.org/abs/1003.1735> (2010).
14. E. Fenu, D. G. Figueroa, R. Durrer, J. García-Bellido, *J. Cosmol. Astropart. Phys.* **10**, 005 (2009).
15. D. Baumann, M. Zaldarriaga, <http://arxiv.org/abs/0901.0958> (2009).
16. P. Adshead, E. A. Lim, <http://arxiv.org/abs/0912.1615> (2009).
17. M. Kamionkowski, A. Kosowsky, A. Stebbins, *Phys. Rev. D* **55**, 7368 (1997).
18. M. Zaldarriaga, U. Seljak, *Phys. Rev. D* **55**, 1830 (1997).
19. W. Hu, M. J. White, *N. Astron.* **2**, 323 (1997).
20. S. Dodelson, *AIP Conf. Proc.* **689**, 184 (2003).
21. M. Zaldarriaga, U. Seljak, *Phys. Rev. D* **58**, 023003 (1998).
22. A. Lewis, A. Challinor, *Phys. Rep.* **429**, 1 (2006).
23. J. Bock *et al.*, <http://arxiv.org/abs/0906.1188> (2009).
24. Planck Collaboration, <http://arxiv.org/abs/astro-ph/0604069> (2006).
25. We acknowledge a host of theoretical and experimental collaborators who have helped focus their energy and interest on new ideas that will play a crucial role in the detection of gravitational waves and whose work we have tried to outline here. Our own research is supported by the U.S. Department of Energy's Office of Science (Arizona), NASA, and the NSF for research at Chicago.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/989/DC1
SOM Text 1 and 2

Table S1
References

10.1126/science.1179541

Adaptive Evolution of an sRNA That Controls *Myxococcus* Development

Yuen-Tsu N. Yu, Xi Yuan, Gregory J. Velicer*

Noncoding small RNA (sRNA) molecules regulate gene expression networks in all domains of life (1, 2), but inferences regarding the roles of sRNAs in adaptive evolution have been indirect. Here, we show that a spontaneous adaptive mutation in the bacterium *Myxococcus xanthus* restored a defective social trait, multicellular fruiting body development, by deactivating a previously unknown sRNA, Pxr. We also show that Pxr acts as a pivotal regulatory checkpoint that blocks *Myxococcus* development until nutrients have been depleted.

Upon starvation, *M. xanthus* cells aggregate, exchange multiple signals, and construct spore-bearing fruiting bodies (3). In a previous study, an *M. xanthus* cheater strain that is defective at development in clonal groups (OC, obligate cheater) evolved into a strain with restored developmental proficiency (PX, phoenix) (4). PX emerged after a cheater-induced population crash during a competition experiment between OC and a developmentally proficient wild-type strain (4). Development was restored in PX by a single C→A mutation in the intergenic region upstream of an acetyltransferase gene (*Mxan_1079*) (fig. S1A) (4). PX development

does not require *Mxan_1079*, because disruption of this gene in strain PX (fig. S2, GJV204, and table S1) does not impair development. Changing the C→A mutation in GJV204 back to the wild-type allele (A→C) abolishes development (fig. S2, GJV205), indicating that the region between *Mxan_1078* and *Mxan_1079* encodes a genetic element, here designated *pxr*, that controls development.

The *pxr* region contains a putative σ^{54} promoter (fig. S1A) preceding a sequence that appears to encode a noncoding sRNA similar in structure to some *Escherichia coli* sRNAs (fig. S1B) (2). The only detected homolog of this sRNA gene was found in the myxobacterium *Stigmatella aurantiaca* (fig. S1B). Two *pxr*-specific sRNA forms, Pxr-L (long) and Pxr-S (short), are present in vegetative cells of the developmentally proficient wild-type strain GJV1 and the defective strain OC (GJV201) but are absent from *pxr*-deletion mutants of GJV1 (GJV206, Fig. 1B) and OC (GJV207, fig. S1C).

Deletion of *pxr* from OC restores development (Fig. 1A, GJV207). Subsequent ectopic expression of the wild-type *pxr*⁺ allele in GJV207 suppressed development (Fig. 1A, GJV208), demonstrating

that Pxr negatively regulates this process. Ectopic expression of the *pxr*^{PX} allele in GJV207 (table S1) failed to block development (Fig. 1A, GJV209), indicating that the C→A mutation in strain PX deactivates Pxr function, thereby allowing development to proceed.

We compared the temporal patterns of Pxr accumulation during starvation in GJV1 and OC (Fig. 1B). Pxr-L is present at high amounts during vegetative growth and starvation in both GJV1 and OC (Fig. 1B). In contrast, Pxr-S is present at high amounts in vegetative cells of both GJV1 and OC but is greatly diminished in GJV1 soon after the onset of starvation (Fig. 1B). This suggests that Pxr-S but not Pxr-L is the primary negative regulator of development when nutrients are abundant and that the rapid reduction of Pxr-S in GJV1 upon starvation allows development to proceed (Fig. 1C). Under this model, OC does not develop because it fails to sufficiently reduce Pxr-S. Indeed, Pxr-S remains present in OC through at least 23 hours of starvation (fig. S3A) but remains absent from GJV1 (fig. S3B). Pxr-S may originate from processing of Pxr-L in a manner analogous to processing of eukaryotic microRNAs (1).

The transition from vegetative growth to development is a critical juncture in the life history of fruiting myxobacteria (5). We tested whether *pxr* might govern this transition in *M. xanthus* by deleting this gene from the wild-type GJV1 and comparing the developmental phenotypes of GJV1 and the resulting mutant (GJV206) at several nutrient (casitone) concentrations (Fig. 1D). GJV1 spore production drops precipitously with increasing casitone, and no spores or fruiting bodies were made at 0.3 to 0.5% casitone. In contrast, GJV206 formed fruiting bodies even at 0.5% casitone and produced nearly as many spores at high casitone concentrations as GJV1 does under complete starvation. These results show that *pxr* is a key developmental gatekeeper that blocks *M. xanthus* development when food is abundant.

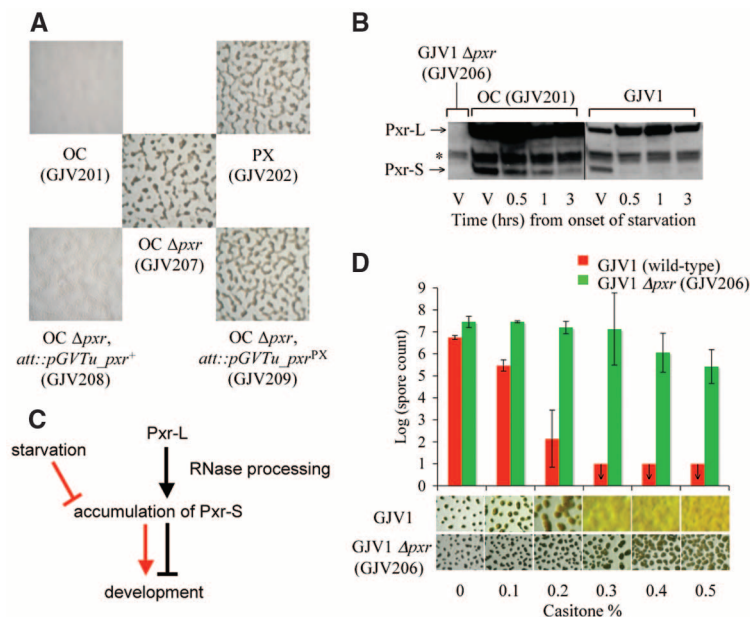


Fig. 1. (A) *pxr* negatively regulates *M. xanthus* development. Images show population phenotypes during starvation. Darkened spots are fruiting bodies containing spores. (B) Temporal patterns of Pxr-L and Pxr-S production in OC and GJV1. V indicates vegetative cells, and the asterisk indicates non-Pxr RNA detected in GJV1 Δ pxr (GJV206). (C) Model of the Pxr developmental checkpoint in *M. xanthus*. Pxr-L and Pxr-S are both produced at high amounts during vegetative growth (black), and Pxr-S blocks development. Upon starvation (red), Pxr-S accumulation is eliminated, and development proceeds. RNase, ribonuclease. (D) *pxr* inhibits development in the presence of nutrients. The graph and images show spore production and developmental phenotypes, respectively, at different casitone concentrations. Arrows indicate zero spores at the limit of detection, and error bars represent 95% confidence intervals.

References and Notes

1. G. Stefani, F. J. Slack, *Nat. Rev. Mol. Cell Biol.* **9**, 219 (2008).
2. S. Gottesman, *Trends Genet.* **21**, 399 (2005).
3. L. J. Shimkets, M. Dworkin, H. Reichenbach, in *The Prokaryotes*, M. Dworkin, Ed. (Springer, New York, 2006), vol. 7, pp. 31–115.
4. F. Fiegna, Y. T. N. Yu, S. V. Kadam, G. J. Velicer, *Nature* **441**, 310 (2006).
5. B. Z. Harris, D. Kaiser, M. Singer, *Genes Dev.* **12**, 1022 (1998).
6. We thank L. Sogaard-Andersen and H.-C. T. Tsui for helpful discussion and C. Fuqua for manuscript suggestions. This work was supported by NIH grant GM079690 to G.J.V.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/993/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
Table S1
References

18 January 2010; accepted 2 April 2010
10.1126/science.1187200

Department of Biology, Indiana University, Bloomington, IN 47405, USA.

*To whom correspondence should be addressed. E-mail: gvelicer@indiana.edu

A Catalog of Reference Genomes from the Human Microbiome

The Human Microbiome Jumpstart Reference Strains Consortium*†

The human microbiome refers to the community of microorganisms, including prokaryotes, viruses, and microbial eukaryotes, that populate the human body. The National Institutes of Health launched an initiative that focuses on describing the diversity of microbial species that are associated with health and disease. The first phase of this initiative includes the sequencing of hundreds of microbial reference genomes, coupled to metagenomic sequencing from multiple body sites. Here we present results from an initial reference genome sequencing of 178 microbial genomes. From 547,968 predicted polypeptides that correspond to the gene complement of these strains, previously unidentified ("novel") polypeptides that had both unmasked sequence length greater than 100 amino acids and no BLASTP match to any nonreference entry in the nonredundant subset were defined. This analysis resulted in a set of 30,867 polypeptides, of which 29,987 (~97%) were unique. In addition, this set of microbial genomes allows for ~40% of random sequences from the microbiome of the gastrointestinal tract to be associated with organisms based on the match criteria used. Insights into pan-genome analysis suggest that we are still far from saturating microbial species genetic data sets. In addition, the associated metrics and standards used by our group for quality assurance are presented.

The human microbiome is the enormous community of microorganisms occupying the habitats of the human body. Different microbial communities are found in each of the varied environments of human anatomy. The aggregate microbial gene tally surpasses that of the human genome by orders of magnitude. Understanding the relationship of the microbial content to human health and disease is one of the primary goals of human microbiome studies. Determining the structure and function of any microbial community requires a detailed definition of the genomes that it encompasses and the prediction and annotation of their genes.

In 2007, the National Institutes of Health (NIH) initiated the Human Microbiome Project (HMP) as one of its Roadmap initiatives (1) to provide resources and build the research infrastructure. One component of the HMP is the production of reference genome sequences for at least 900 bacteria from the human microbiome, which will catalog the microbial genome sequences from the human body and aid researchers conducting human metagenomic sequencing in assigning species to sequences in their metagenomic data sets.

The HMP catalog of reference sequences is being produced by the NIH HMP Jumpstart Consortium of four genome centers: the Baylor College of Medicine Human Genome Sequencing Center, the Broad Institute, the J. Craig Venter Institute, and the Genome Center at Washington University. The challenges for the Jumpstart

Consortium include selecting strains to sequence and identifying sources, creating standards for sequencing and annotation to ensure consistency and quality, and the rapid release of information to the community.

Reference genome progress. To date, 356 genomes, including 117 genomes at various stages of upgrading, have been produced by the Jumpstart Consortium and released into public databases. At the time of manuscript preparation, 178 had been completely annotated and are presented in the analysis here. The process for the selection of these strains is described in (2). The strains sequenced to date are distributed among body sites as follows: gastrointestinal tract (151), oral cavity (28), urogenital/vaginal tract (33), skin (18), and respiratory tract (8). They also include one isolate from blood (3). These are the five major body sites targeted by the HMP.

The broad phylogenetic distribution of the sequenced strains is presented in Fig. 1, which represents a 16S ribosomal RNA (rRNA) overlay of HMP-sequenced genomes on 16S rRNA sequences from cultured organisms with sequenced genomes (4). HMP-sequenced genomes represent two kingdoms (Bacteria and Archaea), nine phyla, 18 classes, and 24 orders. Additional rRNA overlay figures broken down by individual body sites are available in (5).

To obtain high-quality draft genomes and a meaningful gene list, minimum standards were defined for the assembly and annotation of draft genomes. Three reference bacterial genome assemblies were evaluated for efficacy of gene predictions and genome completeness. Based on the analysis, metrics for assembly characteristics and annotation characteristics were defined [for more details, see (2)]. The quality of

HMP genome assemblies is summarized in Table 1 and exceeds the Jumpstart Consortium standards described in (2), with the exception of some genomes produced before the standards were in place.

Genome improvement. As described in (2), there are justifications for upgrading these high-quality draft assemblies. The Jumpstart Consortium has completed initial improvement work on 26 bacterial genomes that differed significantly with respect to GC content and assembly metrics to explore the effort required and resulting benefits (Fig. 2). The average contig N50 increased 3.63-fold, from 109 kb at draft to 396 kb after improvement. *Bacteroides pectinophilus* displays substantial improvement in N50, from 163 kb in the draft sequence to 862 kb after improvement. *Lactobacillus reuteri* illustrates the opposite extreme, with improvement leading to a smaller contig N50 change, 56 kb to 72 kb. As more genomes improve and some graduate to higher levels of improvement, the assembly state or group of states most useful to the HMP scientific goals will be evaluated.

Pan-genome analysis. A bacterial species' pan-genome can be described as the sum of the core genes shared among all sequenced members of the species and the dispensable genes, or those genes unique to one or more strains studied. To start addressing questions about pan-genomes, we identified all species within our sequenced reference genome catalog for which there was more than one sequenced and annotated genome. Of the nine species identified, four of them have five or more annotated genomes that were generated either by the HMP or by external projects publicly available at the National Center for Biotechnology Information (NCBI); five genomes is the minimum number for which a curve can reliably be fit to pan-genome data. These are *L. reuteri*, *Bifidobacterium longum*, *Enterococcus faecalis*, and *Staphylococcus aureus*. The genomic data used for the analysis consisted of both complete and draft genomes, the only requirement being that >90% of the genome be represented in the available annotated contigs or scaffolds.

Pan-genome curves (6) of the gastrointestinal tract isolates *L. reuteri*, *B. longum*, and *E. faecalis* (figs. S3 to S5) are consistent with an open pan-genome model, suggesting that more genome sequencing needs to be undertaken to characterize the actual makeup of the species as a whole. Preliminary results suggest core genome sizes of approximately 1430 genes, 1800 genes, and 1600 genes for *B. longum*, *E. faecalis*, and *L. reuteri*, respectively. Based on the current core gene plots, *L. reuteri* (fig. S3) appears to be approaching a closed pan-genome model, with newly sequenced strains contributing very small numbers of new genes to the pan-genome; however, we see an interesting community substructure within this species. Our current *L. reuteri* pan-genome analysis of seven isolates suggests that four of the

*All authors with their affiliations and contributions are listed at the end of this paper.

†To whom correspondence should be addressed. E-mail: kenelson@jcv.org

seven currently sequenced isolates are very similar to one another, contributing zero to two new genes to the pan-genome. Two further strains are also similar to one another, each contributing an intermediate number of new genes (~15 to 30),

whereas one outlier strain contributes a distinct set of genes (~330). These findings are consistent with the comparison of average nucleotide identity with gene content discussed below for this species. It will be interesting to see whether ad-

ditional sequencing of this species identifies other subgroups in addition to the three identified here, or whether this sample set is in fact largely representative of the species.

Similar findings for *B. longum* (fig. S4) suggest that four of the five currently sequenced genomes contribute approximately equally to the pan-genome (~50 to 150), with one outlier strain (ATCC 15697) contributing a much higher number of previously unidentified (“novel”) genes (~640). These data are consistent with differences in gene count across these genomes. Each of the five currently sequenced genomes of *E. faecalis* (fig. S5) contributes approximately equivalent numbers of new genes to the pan-genome. Our current data sets for these two species are still too small to determine whether we can realistically achieve a closed pan-genome, with newly sequenced isolates contributing on the order of 100 new genes each. It is unrealistic at this point to extrapolate how many additional genomes would need to be sequenced to determine whether the number of new genes contributed by each new sequence continues to plateau around 100 new genes or approaches zero.

S. aureus pan-genome plots (fig. S6), representing isolates collected from the skin, urogenital tract, and mucus membranes of mammals (human and bovine), are consistent with a closed pan-genome model, as previously suggested (7), with an estimated core size of 2295 genes and an estimated pan-genome size of ~3200 genes.

We performed a preliminary survey looking into the functions encoded by those genes that are unique to new gene data sets and not found in the core data set, based on gene product annotation and Enzyme Commission (EC) numbers, when available. These genomes underwent automated annotation only, with no manual curation, so any trends seen should be considered putative. Across all four species, the number of novel genes annotated only as hypothetical or a conserved do-

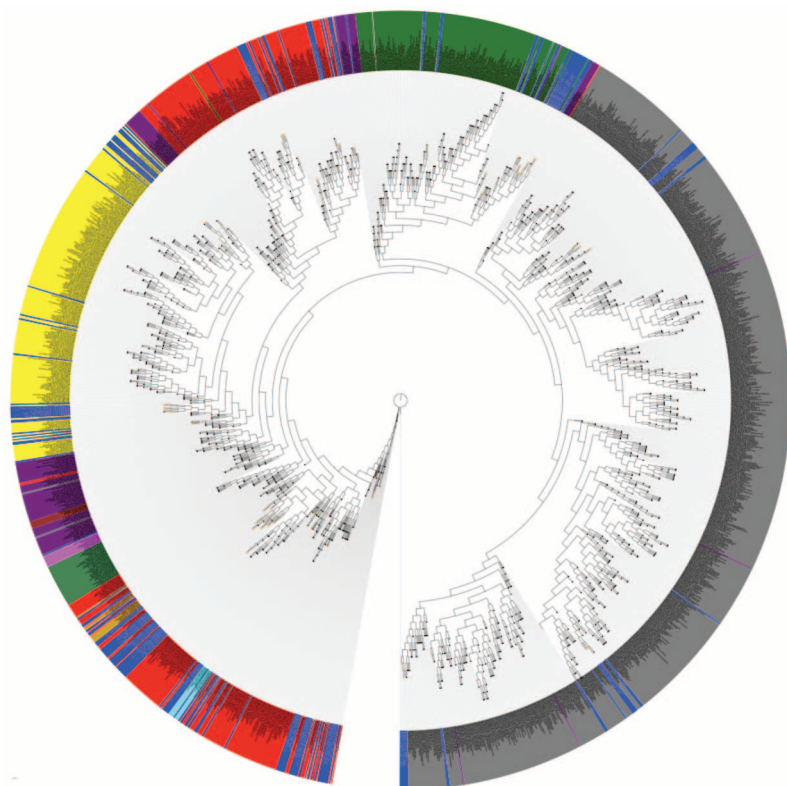


Fig. 1. Phylogenetic tree of 16S rDNA sequences. The tree was created using ~1500 16S rDNAs representing single species. Organisms sequenced as part of the HMP project are highlighted in blue. Additional coloring indicates separation by phylum: yellow, Actinobacteria; dark green, Bacteroidetes; light green, Cyanobacteria; red, Firmicutes; cyan, Fusobacteria; dark red, Planctomycetes; gray, Proteobacteria; magenta, Spirochaetes; light pink, TM7; tan, Tenericutes. The purpose of this analysis is not the details of the branching structure (which include minor known artifacts), but the overall distribution of the HMP strains (in blue) around the tree of life.

Table 1. Draft assembly metrics, organized by finishing status and based on current assignments. Draft corresponds to standard or high-quality draft sequences, with no additional automated or manual attempts to improve assembly, beyond ensuring

exclusion of contaminating sequence. Improved columns correspond to improved high-quality draft submission. None of the reference genomes has been improved beyond this grade at this point. n/a, not applicable.

Metric	Passing standard	Draft Number of strains = 133			Improved Number of strains = 45		
		Pass %	Mean	Range	Pass %	Mean	Range
Percent of genome included in contigs*	>90%	100%	98.23%	95.1–99.9%	100%	99.91%	98.6–100%
Percent of bases greater than 5× read coverage†	>90%	99%	98.90%	80.8–100%	100%	99.35%	98.8–99.6%
Contig N50	>5 kb	100%	102.61 kb	11.12–861.67 kb	100%	517.92 kb	58.03–3472.99 kb
Contig N75	n/a	99%	54.82 kb	4.97–556.76 kb	100%	340.20 kb	30.56–2635.77 kb
Contig N90	n/a	90%	25.54 kb	2.01–240.69 kb	100%	211.51 kb	14.96–2635.77 kb
Scaffold N50*	>20 KB	100%	883.93 kb	50.56–3356.77 kb	100%	606.77 kb	91.71–2898.42 kb
Scaffold N75*	n/a	100%	511.35 kb	24.31–3237.97 kb	100%	378.22 kb	52.32–2391.23 kb
Scaffold N90*	n/a	99%	282.14 kb	11.74–2490.47 kb	100%	226.24 kb	28.67–2391.23 kb
Average contig length	>5 kb	100%	31.52 kb	5.62–180.70 kb	100%	174.70 kb	23.26–1321.04 kb
Percent of core genes present in gene list	>90%	99%	99.63%	86.4–100%	100%	99.90%	98.5–100%

*Calculated only for strains with scaffold assemblies submitted to NCBI. The number of strains with scaffold assemblies, by grade: draft, 74; improved, 37.

†Per-base coverage not available for all reads, for example, those with some draft level of sequencing before the Jumpstart initiative or strains where a combination of technologies was used. The number of strains with per-base read coverage: draft, 121; improved, 4.

main of unknown function ranged from 66 to 73%, making up the bulk of the novel genes identified by the pan-genome analysis. Another predominant trend seen was unique family members corresponding to non-novel functions; for example, functions also identified in the core data set.

There are a number of interesting categories of functions identified in novel gene sets that are unique to individual strains. These include accessory proteins involved in the activation of urease; a virulence factor found in microorganisms associated with gastric ulceration, among other human health concerns (8); phage morphogenesis and regulation proteins; and small numbers of enzymes involved in the metabolism of sugars and amino acids. Further work is needed to clean up annotations and to provide more consistent EC number assignments in order to confirm and build on trends seen in this preliminary analysis. The HMP Data Analysis and Coordination Center (DACC) has been given the mandate of adding value and updating annotations, which will allow for the expansion of these analyses throughout this project.

Measuring diversity within genera. The genomic diversity among strains belonging to the same genus was explored by a measure of the evolutionary relatedness and gene content similarity in a pairwise fashion (Fig. 3). The average nucleotide identity (ANI) is a measure of evolutionary relatedness based on sequence similarity between the set of shared genes (9). The measure of gene content similarity between two strains can provide a sense of functional or ecological relatedness, and one might predict that strains with a lower gene content similarity are more likely to be found in different habitats. The three genera selected for this comparison all contain at least 16 strains and include *Lactobacillus* (36 strains; Fig. 3), *Bifidobacterium* (16 strains; fig. S7), and *Bacteroides* (21 strains; fig. S8). Genomes contributed by the HMP as well as those available in public databases were included in this analysis. High intraspecies diversity was observed within genera in addition to interspecies diversity. Within *Lactobacillus*, several species showed significant diversity. For example, *L. reuteri* is represented by two main groups; one set (bottom left blue oval in Fig. 3) contains seven different strains. Among the strains within that group, the percent of ANI (%ANI) and percent of gene content are above 98 and 90%, respectively. In the second group (upper right blue oval in Fig. 3), the % ANI ranges between 96 and 93%, with a gene content similarity lower than 78%. Previously, a value of 95% ANI was shown to correspond with the recommended cutoff of 70% DNA-DNA reassociation for species delineation (10). This indicates that the *L. reuteri* strains obtained within the framework of the HMP significantly increased the known genomic diversity of this named species, as was also demonstrated by the pan-genome analysis. Other strains showing

large intraspecies diversity belong to *L. johnsonii* and *L. gasseri*.

Among the strains of *B. longum* (fig. S7), four (two of which were contributed by the HMP) have pairwise %ANI values at the higher end of the spectrum, ranging between 96 and 98%, but with relatively low gene-content similarity (that is, below 82%), indicating a broad range in gene complements. One additional existing strain (ATCC 15697) has a %ANI below 95% and a gene content similarity below 65% and is therefore a clear evolutionary and ecological outlier.

The analysis of *Bacteroides* genomes has revealed several close common ancestries. *Bacteroides* sp. D4 and 9_1_42FAA are closely related to *Ba. dorei* (ANI > 95%), but still have a significant gene content difference, lower than 78% similarity. This suggests that the *Bacteroides* group may have many closely related, yet ecologically distinct lineages.

Novel genes. The 547,968 predicted polypeptides corresponding to the entire annotated gene complement of these strains [of which 516,631 (94%) were unique] were searched against the bacterial and viral divisions of NCBI's nonredundant (nr) protein database using WU-

BLASTP as described in (5). Each polypeptide was also compared to a merged database of TIGRFAM and Pfam hidden Markov models (HMMs) using version 2a of the HMMER3 package. A set of candidate novel polypeptides was defined by selecting those that had both of the following conditions: (i) unmasked sequence length >100 amino acids and (ii) no BLASTP match to any nonreference entry in the nr subset. This analysis resulted in a set of 30,867 polypeptides, 5.6% of the total, of which 29,987 (~97%) were unique (2). Clustering this set with CD-HIT (11) resulted in 29,286 unique polypeptides at 98% sequence identity (~5% reduction), 28,857 polypeptides at 95% (~7% reduction), and 28,469 at 90% (~8% reduction). An alternate set of candidate novel polypeptides was also defined by modifying condition (i) above to filter on the number of bases not identified as low-complexity sequences by the SEG algorithm (12) (that is, the sequence length after removing all SEG-masked bases). This alternate initial set contains 28,693 polypeptides.

The above criteria were chosen by inspecting histograms of novel versus non-novel polypeptide counts at various expectation (E)-value and sequence-length thresholds and selecting cutoffs

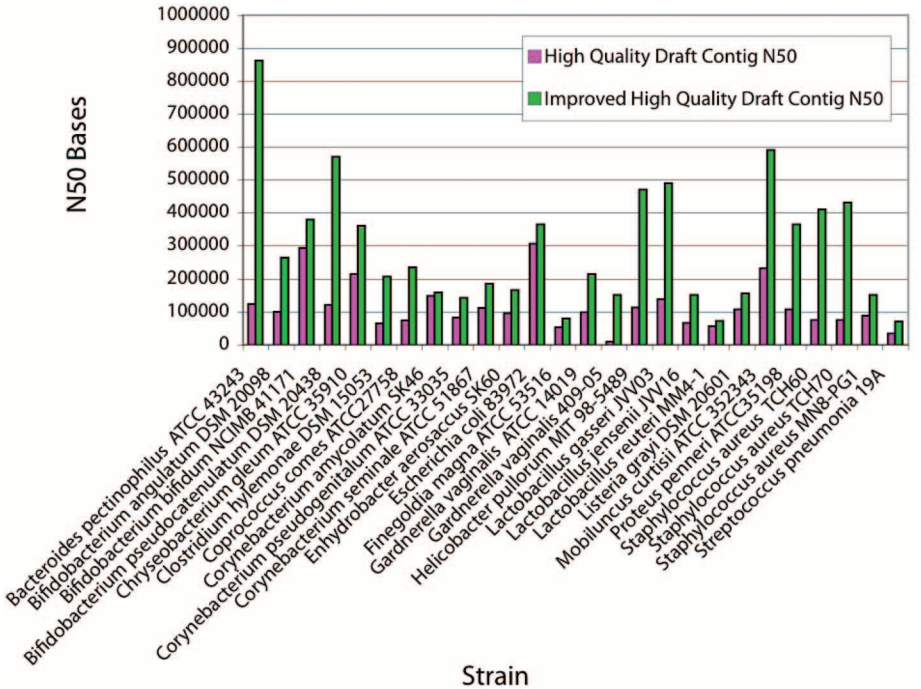


Fig. 2. Contig N50 comparison for 26 draft and improved genomes. High-quality draft contig N50 bases are shown in magenta, and improved high-quality draft sequences are shown in green. These data represent the variety of approaches from the four data-generation centers. The majority of shotgun data was produced on the Roche-454 platform, although some assemblies include paired Sanger reads to improve contiguity. All draft assemblies are based on the Roche-Newbler assembler, although some of the improved assemblies are based on Parallel Contig Assembly Program (PCAP) (23) and the Celera Assembler (24) due to existing integration with finishing and improvement pipelines. Additional variation comes from the improvement approach. Directed Sanger reads from gap-spanning polymerase chain reaction amplicons served as the primary approach, whereas some assemblies have been subjected only to manipulation of the shotgun data, making unrealized joins, removing poor-quality data, and placing unincorporated shotgun reads.

that seemed likely to minimize the number of false positives while not excluding too many true positives. The distribution of novel versus non-novel polypeptide counts overlaps at all E-value thresholds, making it impossible to pick a cutoff that does not exclude any true positives. Therefore a relatively long (100 amino acids) length threshold was selected to try to minimize noise or false positives, at the possible cost of losing some real novel polypeptides.

With ~1300 completely sequenced bacterial genomes in GenBank (13), the observation that 5% of the genes annotated in the HMP genomes satisfy criteria for novelty underscores the remarkable diversity of bacterial proteins. To assess whether there is enriched novelty in the HMP-targeted genomes as compared with previously sequenced prokaryotic genomes, we randomly selected 178 previously sequenced draft genomes from GenBank and ran the same analysis for comparison. This data set resulted in 747,522 predicted polypeptides, of which 568,426 were unique. Of these, 14,269 polypeptides met our criteria for novelty, 1.9% of the total, of which 14,064 were unique, 2.5% of the unique total. Clustering resulted in a 2% reduction at 98% and a 3% reduction at 90%, indicating that this data set does not contain as many highly similar protein predictions as the HMP novel set does. This suggests that there is enrichment in novelty in the HMP data set of approximately 2:1 over the random data set. Whereas the human microbiome is generally thought to be less complex than that of

soils and certain other environmental microbiomes, it nevertheless clearly houses enormous microbial diversity yet to be described.

Analysis of metagenomic shotgun data.

Because the HMP reference genomes that were sequenced had been selected primarily because they were isolates from humans and had not been identified as strains seen in metagenomics studies, it was not known how much these genomes would help to identify metagenomic sequences that were obtained from human microbial communities. The most useful reference genomes should expand our ability to interpret metagenomic data. We also used the stringent fragment recruitment technique (14) to compare metagenomic sequencing data to the reference genomes in nucleotide space (15). The stringency of this approach generally limits the recruitment of metagenomic reads to organisms within the same genus, but it can resolve strain-specific differences.

Publicly available metagenomic data sets from two human gastrointestinal studies were used in this analysis (16, 17), along with 454 reads from a Washington University data set (which contributed the bulk of the 16.8 million reads that were tested). The reference genomes included 866 complete and 913 draft genomes available at NCBI, including the HMP reference genomes with sequence reads available at the time of analysis. In total, 62 HMP genomes showed significant levels of recruitment with 11.3 million metagenomic reads recruited (66% of all reads). Of these, a significant 6.9 million

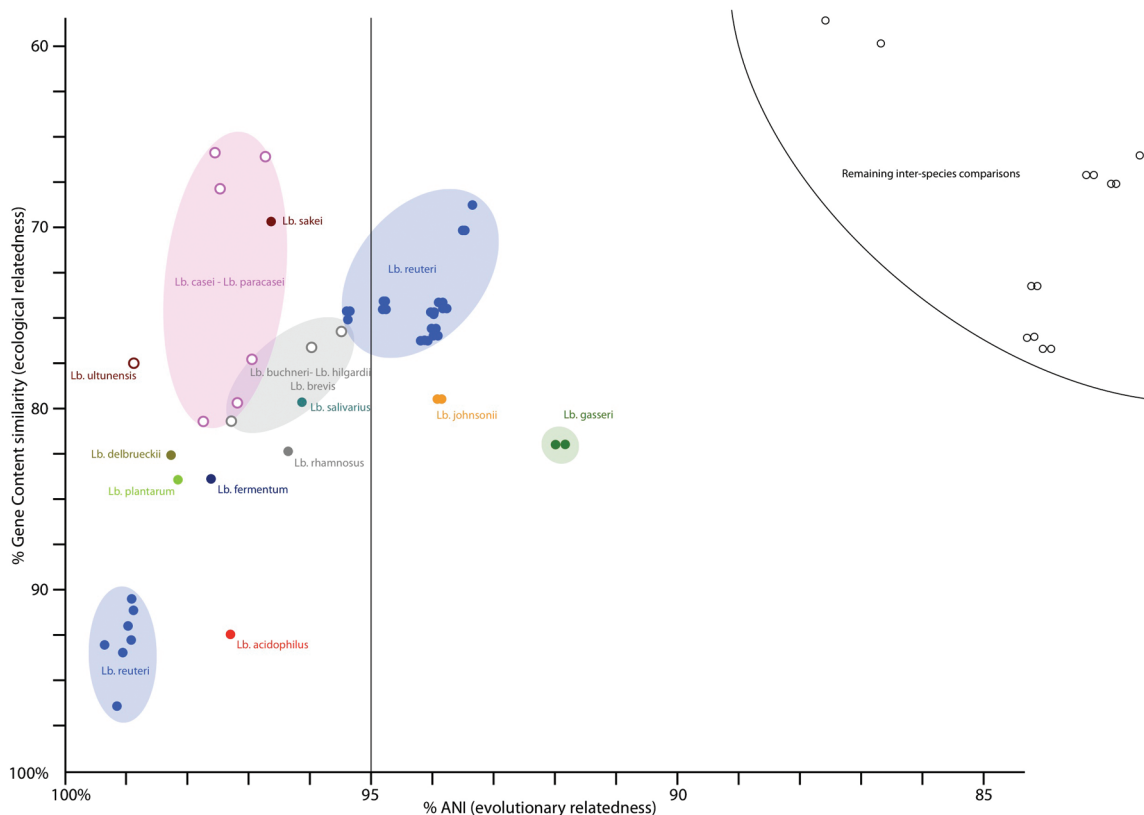
reads (41%) recruited best to the HMP reference genomes, based on the global percent identity (defined as the number of identities between read and reference, divided by the length of the read). A read is considered to be a best hit to a HMP genome if the best global percent identity includes a match to an HMP genome. Many of these reads would not have been recruited if the HMP reference genomes were not available: Between 20 and 40% of the reads were recruited only because of the presence of the HMP genomes.

These results show that a significant number of the genomes sequenced as part of the HMP project are directly adding to our understanding the human microbiome. These results also show that specific genomes are useful references across a wide range of individuals despite the strain-specific diversity noted above. Despite the large number of genomes available, a significant amount of the metagenome (33%) is still not well represented by any reference genome. It is likely that the 900-genomes target of the HMP will reduce this number of unidentified reads further without redundancy in genome selection. It should be noted that this analysis focused on the gastrointestinal tract, and it is likely that additional genomes exist in other body sites; thus, the composition of the 900 genomes should address these organisms.

Data release, future plans, and conclusions.

The Jumpstart Centers have made substantial progress in generating a set of reference genomes that describe the human microbiome. We have

Fig. 3. Interstrain diversity among *Lactobacillus* genomes. Each point represents a whole-genome comparison between two *Lactobacillus* genomes and shows the %ANI on the x axis as a measure of evolutionary distance, plotted against the percentage of gene content similarity on the y axis. Only comparisons with ANI values above 85% are shown. The vertical line at 95% corresponds to a recommended cut-off of 70% DNA-DNA reassociation for species delineation. Different intra- and interspecies comparisons are color coded, with solid or open circles respectively, and labeled with their given taxonomical name in the corresponding color. Colored ovals assist in identifying related data points belonging to a single named species.



made every effort to ensure that all strains are available in public repositories and to release these genomes and their associated data, assemblies, and annotations in accordance with NIH policy (18). In addition, all data and standard operating procedures are available through the DACC (19), where we welcome community input and feedback.

Human microbiome research groups from around the world have launched an International Human Microbiome Consortium (IHMC), which together will sequence more than 1000 human microbial bacterial reference genomes. These include the 900 reference strains that are being sequenced by the HMP Jumpstart Centers, 100 genomes sequenced as part of the European Union-funded MetaHIT project (20), and additional genomes produced by international efforts. All of these strains appear on the DACC. Other strains are being sequenced as part of the Department of Energy Genomic Encyclopedia of Bacteria and Archaea (GEBA) (21, 22) project.

Nevertheless, the human microbiome is much more complex than this set of genomes and is likely to exceed it by orders of magnitude. In addition to the large number of cultured strains, many unculturable strains remain to be defined, and substantial intraspecies diversity still needs to be described. Thus, this initial effort is only a beginning, but it is valuable, and not only does it contribute to the catalog of reference strains, but it also builds infrastructure for strain selection and acquisition, develops methods for sequencing unculturable, defines standards for the various deliverables, provides online access to the large new data set, and addresses many other issues.

The development of standards that will be applied to the 900 genomes that are being sequenced will provide a new and higher level of uniformity to microbial genome data. The Jumpstart Consortium members are also in discussion with other consortia that are interested in standards to extend this uniformity beyond the HMP.

This report and the initial stage of the HMP focus on bacteria, but this effort is currently being expanded to produce reference genomes for eukaryotic microbes and viruses. These other components of the human microbiome have not been forgotten, but the initial focus on bacteria has allowed necessary infrastructure to be developed for the large task ahead, which can now be readily deployed for other organisms. It is our ultimate goal to sample the human microbiome as completely as possible.

References and Notes

1. The NIH Common Fund Human Microbiome Project, Division of Program Coordination, Planning and Strategic Initiatives, NIH, U.S. Department of Health and Human Services, <http://nihroadmap.nih.gov/hmp/>.
2. Materials and methods are available as supporting material on Science Online.
3. HMP Project Catalog, Human Microbiome Project Data Analysis Coordinating Center, www.hmpdacc.org/project_catalog.html.
4. 16S rDNA for cultured bacteria, http://bioinfo.unice.fr/blast/documentation/alphabetical_list.html.
5. Reference genomes of the Human Microbiome Project, Human Microbiome Project Data Analysis Coordinating Center, http://hmpdacc.org/reference_genomes.php.
6. H. Tettelin et al., *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13950 (2005).
7. H. Tettelin, D. Riley, C. Cattuto, D. Medini, *Curr. Opin. Microbiol.* **11**, 472 (2008).
8. H. L. Mobley, M. D. Island, R. P. Hausinger, *Microbiol. Rev.* **59**, 451 (1995).
9. K. T. Konstantinidis, A. Ramette, J. M. Tiedje, *Appl. Environ. Microbiol.* **72**, 7286 (2006).
10. J. Goris et al., *Int. J. Syst. Evol. Microbiol.* **57**, 81 (2007).
11. W. Li, J. C. Wooley, A. Godzik, D. Jones, *PLoS ONE* **3**, e3375 (2008).
12. J. C. Wootton, S. Federhen, *Comput. Chem.* **17**, 149 (1993).
13. National Center for Biotechnology Information, U.S. National Library of Medicine, www.ncbi.nlm.nih.gov/.
14. D. B. Rusch et al., *PLoS Biol.* **5**, e77 (2007).
15. Genome selection page organized by coverage, J. Craig Venter Institute, <http://gos.jcvi.org/users/hmpGenomes/genomes.html>.
16. P. J. Turnbaugh et al., *Nature* **457**, 480 (2009).
17. S. R. Gill et al., *Science* **312**, 1355 (2006).
18. M. Y. Giovanni, *Genome Sequencing Centers NIAID Data and Reagent Sharing and Release Guidelines*, National Institute of Allergy and Infectious Diseases, NIH, U.S. Department of Health and Human Services, www.niaid.nih.gov/dmid/genomes/mscs/data_release.htm.
19. Documentation and SOPs, Human Microbiome Project Data Analysis Coordinating Center, www.hmpdacc.org/sops.php.
20. J. Qin et al., *Nature* **464**, 59 (2010).
21. A Genomic Encyclopedia of Bacteria and Archaea (GEBA), Joint Genome Institute, U.S. Department of Energy Office of Science, www.jgi.doe.gov/programs/GEBA/.
22. D. Wu et al., *Nature* **462**, 1056 (2009).
23. X. Huang, J. Wang, S. Aluru, S. P. Yang, L. Hillier, *Genome Res.* **13**, 2164 (2003).
24. J. Miller et al., *Bioinformatics* **24**, 2818 (2008).
25. The authors gratefully acknowledge J. Warren, J. Zhang, R. G. Fowler, P. Pham, D. Haft, J. Selengut, T. Davidsons, P. Goetz, D. Harkins, S. Shrivastava, S. Koren, B. Walenz, L. Foster, I. Singh, Y.-h. Rogers, and the J. Craig Venter Institute Joint Technology Center. We thank J. Xu, S.-P. Yang, and S. Schobel for bioinformatics support; the Broad Genome Sequencing Platform, Y. Han, V. Korchina, M. Scheel, R. Thornton and the BCM-HGSC production team, L. Courtney, C. Fronick, O. Hall, M. O'Laughlin, M. Cunningham, D. O'Brien, B. Theising, and the GCWU production team for sequencing; J. Gordon, F. Dewhirst, B. Wilson, B. White, R. Mandrell, M. Blaser, R. H. Stevens, S. Hillier, Y. Liu, Z. Shen, D. Schauer, J. Fox, M. Allison, C. D. Sibley, D. M. Saulnier, and G. R. Gibson for providing strains; and M. Y. Giovanni, C. L. Baker, V. Bonazzi, C. D. Deal, S. Garges, R. W. Karp, R. W. Lunsford, J. Peterson, M. Wright, T. T. Belachew, and C. R. Wellington for funding agency management. We acknowledge NIH for funding this project with grants to the J. Craig Venter Institute (grants N01 AI 30071 and U54-AI084844), Washington University (grants U54-HG003079 and U54-HG004968), Baylor College of Medicine (grants U54-HG003273 and U54-HG004973), and the Broad Institute (grants HHSN272200900017C and U54-HG004969). Funding for E.A.-V. was from the Crohn's and Colitis Foundation of Canada; D.G. had secondary affiliation at the Laboratory of Microbiology (WE 10), Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, K. Ledegangstraat 35, 9000 Ghent, Belgium, and is indebted to the Fund for Scientific Research, Flanders (Belgium), for a postdoctoral fellowship and research funding for the duration of this project; M.S. acknowledges the Canadian Cystic Fibrosis Foundation and the Canadian Institutes of Health Research for funding of his research for this project.

The Human Microbiome Jumpstart Reference Strains Consortium

Manuscript preparation: Karen E. Nelson,^{1†} George M. Weinstock,² Sarah K. Highlander,^{3,4} Kim C. Worley,^{3,5} Heather Huot Creasy,⁶ Jennifer Russo Wortman,^{7,6} Douglas B. Rusch,⁸ Makedonka Mitreva,² Erica Sodergren,² Asif T. Chinwalla,² Michael Feldgarden,⁹ Dirk Gevers,⁹ Brian J. Haas,⁹ Ramana Madupu,⁸ Doyle V. Ward.⁹

Principal investigators: Bruce W. Birren,⁹ Richard A. Gibbs,^{3,5} Sarah K. Highlander,^{3,4} Barbara Methe,¹ Karen E. Nelson,¹ Joseph F. Petrosino,^{3,4} Robert L. Strausberg,¹ Granger G. Sutton,⁸ George M. Weinstock,² Owen R. White,^{10,6} Richard K. Wilson.²

Annotation: Asif T. Chinwalla,² Heather Huot Creasy,⁶ Scott Durkin,⁸ Michelle Gwinn Giglio,⁶ Sharvari Gujja,⁹ Brian J. Haas,⁹ Sarah K. Highlander,^{3,4} Clint Howarth,⁹ Chinnappa D. Kodira,¹¹ Nikos Kyrpides,¹² Ramana Madupu,⁸ Teena Mehta,⁹ Makedonka Mitreva,² Donna M. Muzny,^{3,5} Matthew Pearson,⁹ Kymberlie Pepin,² Amrita Pati,¹² Xiang Qin,^{3,5} Kim C. Worley,^{3,5} Jennifer Russo Wortman,^{7,6} Chandri Yandava,⁹ Qiangdong Zeng,⁹ Lan Zhang.^{3,5}

Assembly: Aaron M. Berlin,⁹ Lei Chen,² Theresa A. Hepburn,⁹ Justin Johnson,⁸ Jamison McCarrison,⁸ Jason Miller,⁸ Pat Minx,² Donna M. Muzny,^{3,5} Chad Nusbaum,⁹ Xiang Qin,^{3,5} Carsten Russ,⁹ Granger G. Sutton,⁸ Sean M. Sykes,⁹ Chad M. Tomlinson,² Sarah Young,⁹ Wesley C. Warren,² Kim C. Worley.^{3,5}

Data analysis: Jonathan Badger,¹³ Jonathan Crabtree,⁶ Heather Huot Creasy,⁶ Michael Feldgarden,⁹ Dirk Gevers,⁹ Sarah K. Highlander,^{3,4} Ramana Madupu,⁸ Victor M. Markowitz,¹⁴ Makedonka Mitreva,² Donna M. Muzny,^{3,5} Joshua Orvis,⁶ Joseph F. Petrosino,^{3,4} Douglas B. Rusch,⁸ Granger G. Sutton,⁸ Doyle V. Ward,⁹ Kim C. Worley,^{3,5} Jennifer Russo Wortman.^{7,6}

DNA sequence production: Andrew Cree,^{3,5} Steve Ferriera,¹⁵ Lucinda L. Fulton,² Robert S. Fulton,² Marcus Gillis,¹ Lisa D. Hemphill,^{3,5} Vandita Joshi,^{3,5} Christie Kovar,^{3,5} Donna M. Muzny,^{3,5} Manolito Torralba,¹ Xiang Qin.^{3,5}

Funding agency management: Kris A. Wetterstrand.¹⁶

Genome improvement: Amr Abouelleil,⁹ Aye M. Wollam,² Christian J. Buhay,^{3,5} Yan Ding,^{3,5} Shannon Dugan,^{3,5} Michael G. Fitzgerald,⁹ Lucinda L. Fulton,² Robert S. Fulton,² Mike Holder,^{3,5} Jessica Hostetter,¹ Ramana Madupu,⁸ Donna M. Muzny,^{3,5} Xiang Qin,^{3,5} Granger G. Sutton.⁸

Project leadership: Bruce W. Birren,⁹ Sandra W. Clifton,² Sarah K. Highlander,^{3,4} Karen E. Nelson,¹ Joseph F. Petrosino,^{3,4} Erica Sodergren,² Robert L. Strausberg,¹ Granger G. Sutton,⁸ George M. Weinstock,² Owen R. White.^{10,6}

Strain management: Emma Allen-Vercor,¹⁷ Jonathan Badger,¹³ Sandra W. Clifton,² Heather Huot Creasy,⁶ Ashlee M. Earl,⁹ Candace N. Farmer,² Michelle Gwinn Giglio,⁶ Marcus Gillis,¹ Sarah K. Highlander,^{3,4} Konstantinos Liolios,¹² Karen E. Nelson,¹ Erica Sodergren,² Michael G. Surette,¹⁸ Granger G. Sutton,⁸ Manolito Torralba,¹ Doyle V. Ward,⁹ George M. Weinstock,² Jennifer Russo Wortman,^{7,6} Qiang Xu.¹⁹

Submissions: Asif T. Chinwalla,² Craig Pohl,² Scott Durkin,⁸ Granger G. Sutton,⁸ Katarzyna Wilczek-Boney,^{3,5} Dianhui Zhu.^{3,5}

¹Human Genomic Medicine, J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, USA. ²The Genome Center, Washington University School of Medicine, 4444 Forest Park Avenue, St. Louis, MO 63108, USA. ³Human Genome Sequencing Center, Baylor College of Medicine, BCM226, One Baylor Plaza, Houston, TX 77030, USA. ⁴Department of Molecular Virology and Microbiology, BCM280, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA. ⁵Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA. ⁶Institute for Genome Sciences, University of Maryland School of Medicine, 801 West Baltimore Street, Baltimore, MD 21201, USA. ⁷Department of Medicine, University of Maryland School of Medicine, Department of Genetics, 801 West Baltimore Street, Baltimore, MD 21201, USA. ⁸Bioinformatics, J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, USA. ⁹Genome Sequencing and Analysis Program, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142, USA. ¹⁰Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, 801 West Baltimore

Street, Baltimore, MD 21201, USA. ¹¹Genome Sequencing and Analysis Program, 454 Sequencing, 15 Commercial Street, Branford, CT 06405, USA. ¹²Department of Energy-Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA. ¹³Microbial and Environmental Genomics, J. Craig Venter Institute, 10355 Science Center Drive, La Jolla, CA 92037, USA. ¹⁴Biological Data Management and Technology Center, Lawrence Berkeley National Laboratory, Berkeley, CA

94720, USA. ¹⁵Sequencing, J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, USA. ¹⁶National Human Genome Research Institute, 5635 Fishers Lane, Bethesda, MD 20892, USA. ¹⁷Molecular and Cellular Biology, University of Guelph, 50 Stone Road, Guelph, Ontario N1G 2W1, Canada. ¹⁸Microbiology and Infectious Diseases, University of Calgary, 3330 Hospital Drive, Calgary, Alberta T2N4N1, Canada. ¹⁹Osel Inc., 4008 Burton Drive, Santa Clara, CA 95054, USA.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/994/DC1
Materials and Methods
Figs. S1 to S8
References and Notes

20 October 2009; accepted 31 March 2010
10.1126/science.1183605

REPORTS

Observation of Plasmarons in Quasi-Freestanding Doped Graphene

Aaron Bostwick,¹ Florian Speck,² Thomas Seyller,² Karsten Horn,³ Marco Polini,^{4*} Reza Asgari,^{5*} Allan H. MacDonald,⁶ Eli Rotenberg^{1†}

A hallmark of graphene is its unusual conical band structure that leads to a zero-energy band gap at a single Dirac crossing point. By measuring the spectral function of charge carriers in quasi-freestanding graphene with angle-resolved photoemission spectroscopy, we showed that at finite doping, this well-known linear Dirac spectrum does not provide a full description of the charge-carrying excitations. We observed composite "plasmaron" particles, which are bound states of charge carriers with plasmons, the density oscillations of the graphene electron gas. The Dirac crossing point is resolved into three crossings: the first between pure charge bands, the second between pure plasmaron bands, and the third a ring-shaped crossing between charge and plasmaron bands.

Electrons in metals and semiconductors undergo many complex interactions, and most theoretical treatments make use of the quasiparticle approximation, in which independent electrons are replaced by electron- and hole-like quasiparticles interacting through a dynamically screened Coulomb force. The details of the screening are determined by the valence band structure, but the band energies are modified by the screened

interactions. A complex self-energy function describes the energy and lifetime renormalization of the band structure resulting from this interplay.

Bohm and Pines (*1*) accounted for the short-range interactions between quasiparticles through the creation of a polarization cloud formed of virtual electron-hole pairs around each charge carrier, screening each from its neighbors. The long-range interactions manifest themselves through plasmons,

which are collective charge density oscillations of the electron gas that can propagate through the medium with their own band-dispersion relation. These plasmons can in turn interact with the charges, leading to strong self-energy effects. Lundqvist predicted the presence of new composite particles called plasmarons, formed by the coupling of the elementary charges with plasmons (*2*). Their distinct energy bands should be observable with the use of angle-resolved photoemission spectroscopy (ARPES), but so far have been observed only by optical (*3, 4*) and tunneling spectroscopies (*5*), which probe the altered density of states.

¹Advanced Light Source (ALS), E. O. Lawrence Berkeley Laboratory, MS6-2100, Berkeley, CA 94720, USA. ²Lehrstuhl für Technische Physik, Universität Erlangen-Nürnberg, Erwin-Rommel-Strasse 1, 91058 Erlangen, Germany. ³Department of Molecular Physics, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4-6, 14195 Berlin, Germany. ⁴National Enterprise for nanoScience and nanoTechnology, Istituto Nanoscienze-Consiglio Nazionale della Ricerche and Scuola Normale Superiore, I-56126 Pisa, Italy. ⁵School of Physics, Institute for Research in Fundamental Sciences, Tehran 19395-5531, Iran. ⁶Department of Physics, University of Texas at Austin, 1 University Station C1600, Austin, TX 78712, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: erotenberg@lbl.gov

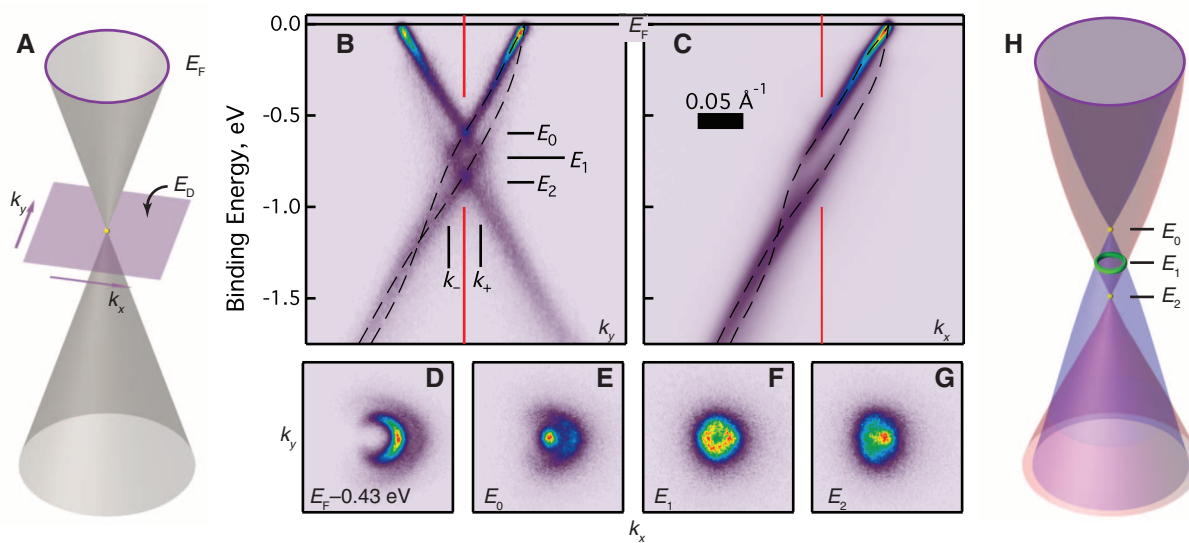


Fig. 1. (A) The Dirac energy spectrum of graphene in a non-interacting, single-particle picture. (B and C) Experimental spectral functions of doped graphene perpendicular and parallel to the Γ K direction of the graphene Brillouin zone. The dashed lines are guides to the dispersion of the observed hole and plasmaron bands. The red lines are at $k = 0$ (the K point of the

graphene Brillouin zone). (D to G) Constant-energy cuts of the spectral function at different binding energies. (H) Schematic Dirac spectrum in the presence of interactions, showing a reconstructed Dirac crossing. The samples used for (B) to (G) were doped to $n = 1.7 \times 10^{13} \text{ cm}^{-2}$. The scale bar in (C) defines the momentum length scale in (B) to (G).

Understanding the coupling between electrons and plasmons is important because of new “plasmonic” devices that have been proposed to merge photonics and electronics. Graphene in particular has been proposed as a promising candidate for such devices (6, 7). Plasmarons have been predicted to occur in graphene and to be observable with ARPES (8, 9), yet their detailed dispersion and interaction with defects remain unknown. Here we present a systematic ARPES study of doped graphene, which reveals details of the plasmaronic band structure.

In a noninteracting picture of graphene, the dispersion relation between the carriers’ energy ω and their momentum $\mathbf{k}$ is expressed by the equation $\omega_b = v_F k$, where v_F is the Fermi velocity of

the carriers and k is the absolute magnitude of $\mathbf{k}$ (10). Here the subscript “b” reminds us that this is the “bare” dispersion. The bands cross at a single point in momentum space ($k = 0$), the so-called Dirac crossing energy E_D as shown in Fig. 1A. Because of the symmetry of graphene’s honeycomb lattice, the spectrum is gapless, and the fundamental particles are effectively massless Dirac fermions. This gives rise to a chiral degree of freedom represented by a pseudospin vector.

We now show that in reality, the topology of the bands near the Dirac crossing differs substantially from this well-known picture. Figure 1B shows the energy band structure of *n*-doped quasi-freestanding graphene, grown epitaxially on H-terminated SiC by a newly described method

(11, 12), chemically doped with potassium atoms (13), and measured with ARPES. Instead of a pair of bands crossing at the Dirac energy E_D , we find four bands, two of them highlighted by dashed lines (the other two are equivalent by reflection across $k = 0$). The data reveal a rearrangement of states around E_D from a single point crossing into a diamond-like shape, characterized by three energies E_0 , E_1 , and E_2 at its apex, middle, and bottom, and two momenta $k_{\pm}$ defining its width. Acquiring the spectrum in a different geometry we can determine the band structure projected onto a single pseudospin orientation (14, 15), as shown in Fig. 1C. This shows that the two highlighted bands have the same chirality. This is confirmed by examining constant-energy cuts of the

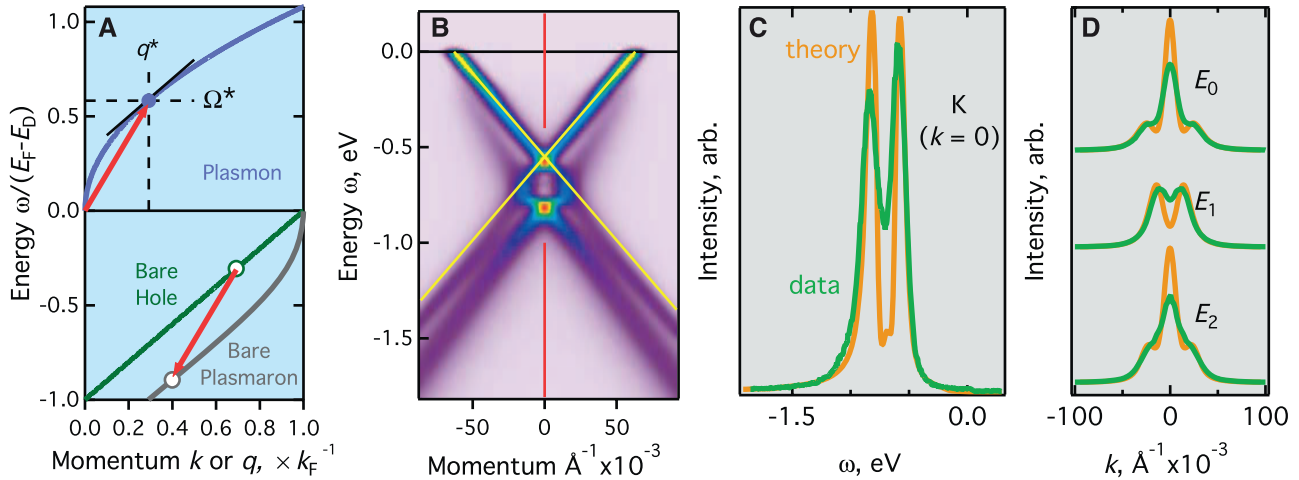


Fig. 2. (A) Comparison of plasmon dispersion function $\Omega(q)$ (top) and bare hole and bare plasmaron quasiparticle dispersions (bottom). The red arrow defines the energy and momentum shifts between plasmaron and hole bands, ignoring hole-plasmon binding. (B) Predicted spectral function according to G_0W -RPA theory.

The yellow lines indicate the bare band structure in the absence of interactions. (C and D) Comparison of the predicted and experimental spectra along different cuts of constant momentum and energy, respectively. In (D), the experimental cuts have been averaged over all azimuths about $k = 0$.

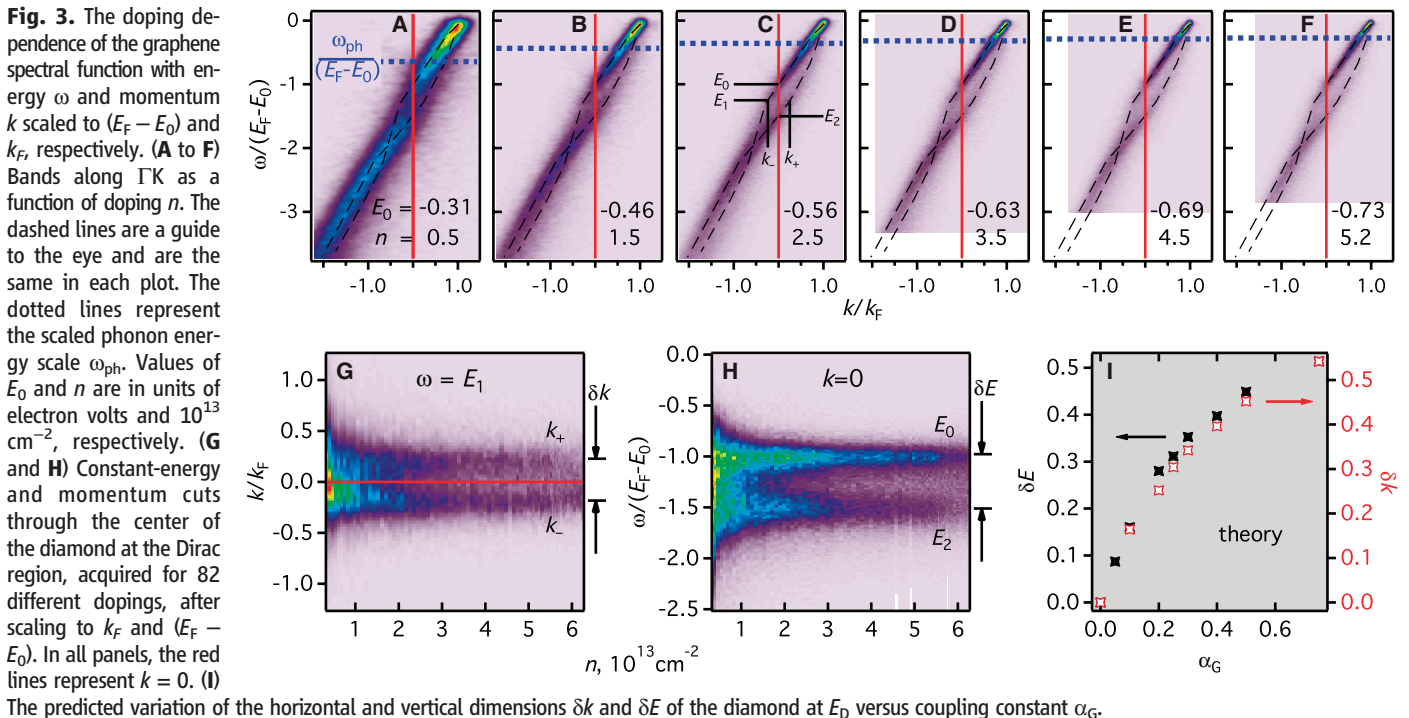


Fig. 3. The doping dependence of the graphene spectral function with energy ω and momentum k scaled to $(E_F - E_D)$ and k_F , respectively. (A to F) Bands along ΓK as a function of doping n . The dashed lines are a guide to the eye and are the same in each plot. The dotted lines represent the scaled phonon energy scale ω_{ph} . Values of E_0 and n are in units of electron volts and 10^{13} cm^{-2} , respectively. (G and H) Constant-energy and momentum cuts through the center of the diamond at the Dirac region, acquired for 82 different dopings, after scaling to k_F and $(E_F - E_D)$. In all panels, the red lines represent $k = 0$. (I) The predicted variation of the horizontal and vertical dimensions δk and δE of the diamond at E_D versus coupling constant α_G .

spectral function (Fig. 1, D to G), which show two bands having identical intensity distributions about the center. The crossing of bands of opposite chirality implies the existence of two Dirac crossings at E_0 and E_2 (Fig. 1, E and G) and a ring-like crossing at energy E_1 , which is resolved in Fig. 1F.

These results show that the single-particle picture in Fig. 1A fails to account for the self-energy arising from interactions between a single charge carrier and the sea of electrons forming a gas in graphene. These data can be better explained with a different model of the electronic structure, as illustrated in Fig. 1H.

In photoemission, a soft x-ray photon promotes an electron from the occupied bands into a free state that is detected by an electron spectrometer, leaving a hole behind. The renormalization of the photohole's lifetime and mass alters the single-particle spectral function $A(\mathbf{k}, \omega)$ of the excited photohole measured by ARPES. Here $\mathbf{k}$ is the photohole momentum and $\omega < 0$ its binding energy. The spectral function $A(\mathbf{k}, \omega)$ is related to the complex self-energy $\Sigma(\mathbf{k}, \omega)$ and the bare band $\omega_b(\mathbf{k})$ through the equation

$$A(\mathbf{k}, \omega) = \frac{\pi^{-1} |\text{Im}\Sigma(\mathbf{k}, \omega)|}{(\omega - \omega_b(\mathbf{k}) - \text{Re}\Sigma(\mathbf{k}, \omega))^2 + (\text{Im}\Sigma(\mathbf{k}, \omega))^2} \quad (1)$$

When the first term in the denominator is zero, a resonance is found in $A(\mathbf{k}, \omega)$ that reflects the existence of quasiparticles having a finite lifetime and an energy only slightly shifted from $\omega_b(\mathbf{k})$. The self-energy arising from short-range interactions (mediated by electron-hole pair generation) is typically small in metals, and the lifetime decreases smoothly as the hole increases in energy, so that

the band dispersion is only slightly different from that of the bare band.

In contrast, the full self-energy function $\Sigma(\mathbf{k}, \omega)$ can have a more complicated energy dependence, leading to multiple resonances at a given $\mathbf{k}$. When this occurs, it signals the emergence of new charged entities that can propagate with well-defined momenta. In one-dimensional metals, for example, this can lead to the breakup of a hole into separately observable spin- and charge-carrying bands, through so-called spin-charge separation (16). For two or more dimensions, new composite particles such as polarons (hole + phonon) (17) or Kondo resonances (localized hole + screening cloud) (18) may be observed. In like manner, for graphene, we attribute the lower band (toward greater binding energy) in Fig. 1 to a distinct quasiparticle reflecting the formation of plasmarons (2), which are composite particles consisting (at a particular momentum $\mathbf{k}$) of holes with momentum $\mathbf{k} + \mathbf{q}$ strongly coupled to plasmons of momentum $-\mathbf{q}$.

This new plasmaronic quasiparticle appears at greater binding energy because of the extra energy cost of creating a plasmon with a hole, which then interact to form the plasmaron. Because the bare plasmon and bare hole energies depend on momentum, this description suggests a broad distribution of states toward higher binding energy, not a discrete quasiparticle. The sharpness of the plasmaronic quasiparticle arises for two reasons: first, because scattering a hole from a plasmon requires conservation of pseudospin as well as momentum, which favors $\mathbf{k}$ and $\mathbf{q}$ to be parallel. The excitation spectrum thus becomes effectively one-dimensional, strengthening the interactions. Second, only plasmons and holes with the same group velocity are likely to form plas-

marons (19), further reducing the phase space for hole-plasmon interactions.

The kinematics of the bare plasmaronic band formation—that is, the formation of the plasmon plus hole without considering their mutual binding interaction—is shown in Fig. 2A. Comparing the plasmon dispersion function $\Omega(q)$ (9, 20, 21) to the bare band structure $\omega_b(k)$, we see that although all holes have the same group velocity (given by the slope of the linear band), only one characteristic plasmon at a single momentum q^* and energy Ω^* (indicated by the red arrow) has the same group velocity as all of the holes and is therefore favored to form the plasmaronic quasiparticle. Except near E_F , the bare plasmaronic band is offset from the bare hole band by approximately these momentum and energy transfers q^* and energy Ω^* . Near E_F , the kinematic restrictions reduce the possible energies and momenta of the interacting plasmon, causing a merging of a much-dampened plasmaron toward the hole band as $k \rightarrow k_F$.

This simple model qualitatively predicts the position and shape of the bare plasmaronic band, but because it neglects the plasmon-hole binding, it underestimates the separation of the plasmaron and hole bands by almost a factor of 2. The actual shift is better represented by detailed calculations of the graphene spectral function (Eq. 1) within the G_0W approximation (8, 9). This approach approximates the self-energy to lowest order by a renormalized Green's function G in which the self-energy is expanded to first order in the dynamically screened Coulomb interaction W computed within the random phase approximation (RPA). An additional phenomenological self-energy arising from electron-phonon coupling is incorporated to account for the well-known band sharpening and kink near $E_F - 180$ meV (13). The computed spectral function is broadened to account for the instrumental resolutions (0.025 eV and $0.01 \text{ \AA}^{-1}$). Figure 2B shows the resulting spectral function, which is in good qualitative agreement with the experimental data in Fig. 1, B and C. These calculations agree well with our observation that the single Dirac crossing at E_D (indicated by the yellow, bare bands) is reconstructed into a diamond-shaped feature with apex energy $E_0 \approx E_D$.

These calculations describe the spectral function around the renormalized Dirac crossing quite well quantitatively, as determined by comparing experimental and theoretical cuts of the spectral function (Fig. 2, C and D) along the energy and momentum directions. The intensity of the plasmaron band in the experimental data is comparable to the theoretical prediction, demonstrating that neither the defect scattering rate nor the symmetry-breaking potential induced by the substrate introduce important energy scales. Both of these effects have been predicted to quickly dampen the plasmaron mode (8, 9, 22).

The doping dependence of the spectral function, presented in Fig. 3, confirms a key prediction of the G_0W -RPA theory, namely that the spectral function should scale with the Fermi energy E_F (measured with respect to E_D) and the

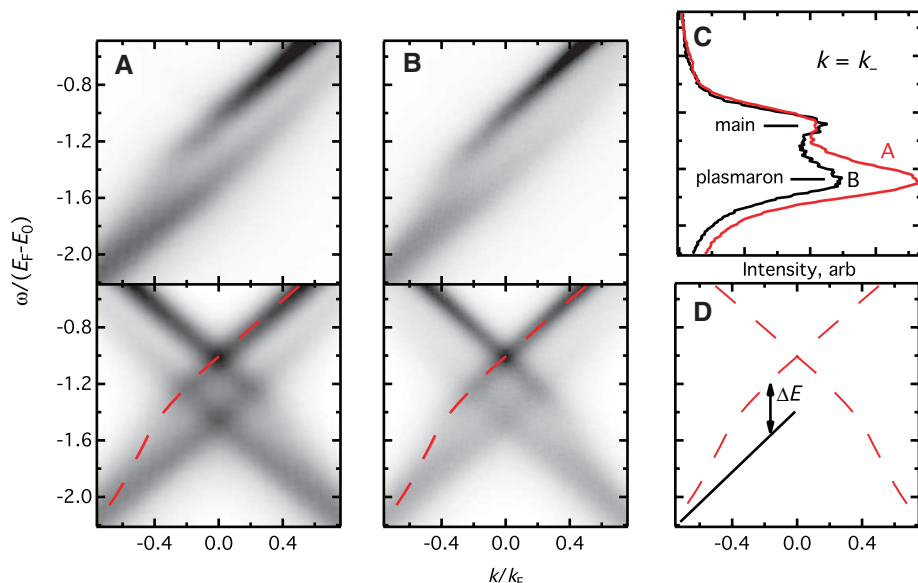


Fig. 4. (A and B) Magnified view of the raw spectral function along the ΓK direction of the graphene Brillouin zone (top) and mirror-symmetrized (bottom) for two different dopings ($n = 0.7$ and $3.3 \times 10^{13} \text{ cm}^{-2}$, respectively). The dashed guide lines are the same in (A), (B), and (D). (C) Energy spectra at $k = k_\perp$ for the same dopings, scaled to the height of the main peak in arbitrary units. (D) The band shift ΔE , which characterizes the dispersion of the bands, is unaltered by defect scattering.

Fermi momentum k_F . This is a special consequence of the linear band structure of graphene and the Coulomb interaction and follows from the linear scaling of the RPA dielectric function (and therefore the plasmon dispersion) with E_F and k_F . [The bare Dirac energy E_D is unknown, so we have equivalently used $(E_F - E_0)$ to scale the data.] These data (Fig. 3, A to F) show that over nearly an order of magnitude variation of charge density, the renormalized bands have indistinguishable shape. Furthermore, when cuts of the measured spectral function at $E = E_1$ and $k = 0$ are plotted with the same scaling, the width $\delta k = |k_+ - k_-|/k_F = 0.38$ and height $\delta E = |E_2 - E_0|/(E_F - E_0) = 0.48$ of the diamond-shaped region are shown to be constants (Fig. 3, G and H) and can therefore be characterized by a single parameter as discussed below. Only at the lowest doping, where the fixed phonon energy scale ($\omega_{ph} \sim 180$ meV) approaches $(E_F - E_0)$ does any deviation appear, evidence of electron-plasmon-phonon mixing.

The parameter that describes the interaction strength is the graphene effective coupling constant $\alpha_G = e^2/\epsilon \hbar v_F \sim 2.2/\epsilon$ (10) (ϵ , extrinsic dielectric constant; $\hbar$, Planck's constant h divided by 2π). [Note that α_G , an intrinsic parameter of the electron-electron interactions within graphene, is to be distinguished from the vacuum coupling constant $\alpha = e^2/\hbar c = 1/137$ determined by optical studies of graphene that characterizes the free-space Coulomb interaction (23, 24).] The dielectric constant $\epsilon = (\epsilon_a + \epsilon_b)/2$ is the average screening contribution of any dielectrics above (ϵ_a) and below (ϵ_b) the graphene. A series of calculations was performed for different values of α_G , and from these calculations (fig. S1), values for δE and δk versus α_G were extracted (Fig. 3I). Comparing to our measurements, we conclude that the best fit is for $\alpha_G \sim 0.5$. From this value, we determine the average screening $\epsilon \sim 4.4$, corresponding to substrate screening contribution $\epsilon_b \sim 7.8$ for graphene on H-SiC in vacuum.

The impact of disorder on the plasmaron bands is revealed by the doping dependence of the spectral weight distribution. This is because the dopant ions themselves can act as weak scatterers (13). Figure 4, A and B show magnified views of the Dirac crossing region for two different dopings. We find that at low doping, the intensity of the plasmaron band is stronger than that of the main band, but at higher doping, the plasmaron band is reduced in strength, in accord with predictions (8, 9). This is evident in the images in Fig. 4 as well as in energy cuts taken at $k = k_-$, at one side of the diamond region (Fig. 4C) and at $k = 0$ (Fig. 3H). (This weakening of the plasmaron band is a characteristic of chemical doping and is not expected to appear in samples doped by applied gate voltage.)

Although the plasmaron mode is dampened by defect scattering, the main band dispersion (indicated by the dashed lines in Fig. 4) remains unaltered. So we can expect that even with heavy damping of the plasmaron bands by disorder, the main bands will still be characterized by a kink in

the Dirac crossing region. This kink reflects the offset $\Delta E \sim (E_2 - E_0)$ between upper and lower π bands illustrated in Fig. 4D.

In fact, such an energy shift ΔE is readily observed for ordinary epitaxial graphene samples (13) prepared by annealing SiC(0001) surfaces in ultra-high vacuum (25) or in inert atmospheres at high temperature (26), although its interpretation in terms of electronic interactions has been controversial. Ordinary epitaxial graphene samples consist of two carbon layers: an insulating buffer layer that saturates the substrate's Si dangling bonds and a second, decoupled layer that acts as single-layer graphene (27). Such samples have a high doping concentration ($n = 1.1 \times 10^{13}$ e/cm²) caused by charge transfer from the substrate and also have weak satellite bands (13) and rippling (28, 29) that arise from the mismatch between the graphene and substrate lattices. There is also a certain degree of disorder at the graphene-SiC interface (30) whose nature is not fully understood.

Comparing the kink at E_D between ordinary graphene [see, for example, figure 1d of (13)] and quasi-freestanding graphene in Fig. 4D, we find a qualitatively similar but smaller shift ΔE at a given doping level in the former. From the obvious reduction of ΔE as $\alpha_G \rightarrow 0$ (fig. S1), we conclude that the effective dielectric constant ϵ was ~ 3 to 4 times larger in the previous samples than in the quasi-freestanding samples. (This indicates that the interfacial buffer layer and dangling bonds are quite polarizable.) For such high screening, the plasmaron band is not resolvable but appears only as a shoulder on the main band, and it is likely to be dampened by the disorder in those samples.

The quantitative agreement between our measurements for quasi-freestanding samples and the theoretical model is satisfying, but we note some deviations between theory and data in Figs. 1 and 2. At higher binding energies, the model predicts distinct electronic and plasmonic bands, but the experiment shows a merging or, as we suggest, a crossing of these bands. These deviations between theory and experiment cannot be explained by lattice effects (31) and instead hint at physics beyond that contained in the G_0W -RPA theory. In this context, graphene can be a good platform for tests of such theories.

For sufficiently high charge density [such that $(E_F - E_D) > k_B T$] ($k_B T$, Boltzmann's constant times temperature), the equilibrium transport properties will be only modestly affected by the interactions we have seen. This is because transport involves carriers within $k_B T$ of k_F , where the plasmaron mode merges to the main band while it is strongly dampened (although not completely suppressed). Nevertheless, our results do establish that the dynamics of a hot population of holes injected into n-type graphene (or similarly, electrons injected into p-type graphene) will be subject to the strong plasmon coupling we have observed. These dynamics might be exploited in new devices (6, 7), such as terahertz amplifiers and plasmon lasing.

In the low-doping limit [$k_B T > (E_F - E_D)$], on the other hand, carriers on the Fermi energy

scale are thermally excited and participate in transport (32). In this weakly doped regime, the RPA theory, which assumes that quasiparticle interactions are weak as compared to the Fermi energy, is least reliable. As we have shown, the Dirac crossing spectrum is reconstructed in a highly nontrivial way even when interactions are weak. Further exploration of this regime at higher temperatures or, equivalently provided that disorder is weak, at lower densities, may reveal more surprises in the future.

References and Notes

- D. Bohm, D. Pines, *Phys. Rev.* **92**, 609 (1953).
- B. Lundqvist, *Phys. Kondens. Materie* **6**, 193 (1967).
- J. L. Shay, W. D. Johnston, E. Buehler, J. H. Wernick, *Phys. Rev. Lett.* **27**, 711 (1971).
- R. Tediosi, N. P. Armitage, E. Giannini, D. van der Marel, *Phys. Rev. Lett.* **99**, 016406 (2007).
- O. E. Dial, thesis, Massachusetts Institute of Technology, Cambridge, MA (2007).
- V. Ryzhii, *Jpn. J. Appl. Phys.* **45**, L923 (2006).
- F. Rana, *IEEE Trans. NanoTechnol.* **7**, 91 (2008).
- E. H. Hwang, S. Das Sarma, *Phys. Rev. B* **77**, 081412 (2008).
- M. Polini *et al.*, *Phys. Rev. B* **77**, 081411 (2008).
- A. H. C. Neto, F. Guinea, N. M. R. Peres, K. S. Novoselov, A. K. Geim, *Rev. Mod. Phys.* **81**, 109 (2009).
- C. Riedl, C. Coletti, T. Iwasaki, A. A. Zakharov, U. Starke, *Phys. Rev. Lett.* **103**, 246804 (2009).
- F. Speck *et al.*, paper presented at the 13th International Conference on Silicon Carbide and Related Materials, Nuremberg, Germany, 11 to 16 October 2009.
- A. Bostwick, T. Ohta, T. Seyller, K. Horn, E. Rotenberg, *Nat. Phys.* **3**, 36 (2007).
- A. Bostwick *et al.*, *New J. Phys.* **9**, 385 (2007).
- M. Mucha-Kruczyński *et al.*, *Phys. Rev. B* **77**, 195403 (2008).
- B. J. Kim *et al.*, *Nat. Phys.* **2**, 397 (2006).
- L. Perfetti *et al.*, *Phys. Rev. Lett.* **87**, 216404 (2001).
- A. Sekiyama *et al.*, *Nature* **403**, 396 (2000).
- P. von Allmen, *Phys. Rev. B* **46**, 13345 (1992).
- E. H. Hwang, S. Das Sarma, *Phys. Rev. B* **75**, 205418 (2007).
- Y. Liu, R. F. Willis, K. V. Emtsev, T. Seyller, *Phys. Rev. B* **78**, 201403 (2008).
- A. Qaiumzadeh, R. Asgari, *New J. Phys.* **11**, 095023 (2009).
- R. Nair *et al.*, *Science* **320**, 1308 (2008).
- A. B. Kuzmenko, E. van Heumen, F. Carbone, D. van der Marel, *Phys. Rev. Lett.* **100**, 117401 (2008).
- I. Forbeaux, J. M. Themlin, J. M. Debever, *Phys. Rev. B* **58**, 16396 (1998).
- K. V. Emtsev *et al.*, *Nat. Mater.* **8**, 203 (2009).
- K. V. Emtsev, F. Speck, T. Seyller, L. Ley, J. D. Riley, *Phys. Rev. B* **77**, 155303 (2008).
- S. Kim, J. Ihm, H. J. Choi, Y.-W. Son, *Phys. Rev. Lett.* **100**, 176802 (2008).
- F. Varchon, P. Mallet, J. Y. Veuillen, L. Magaud, *Phys. Rev. B* **77**, 235412 (2008).
- V. Brar *et al.*, *Appl. Phys. Lett.* **91**, 122102 (2007).
- A. Hill, S. A. Mikhailov, K. Zeigler, *Europhys. Lett.* **87**, 27005 (2009).
- O. Vafek, *Phys. Rev. Lett.* **97**, 266406 (2006).
- The ALS is supported by the director of the Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under contract DE-AC02-05CH11231. Work in Erlangen was supported by the Deutsche Forschungsgemeinschaft through research grant SE1087/5-1 and the Cluster of Excellence "Engineering of Advanced Materials" at the University of Erlangen-Nuremberg. A.H.M. was supported by the Welch Foundation and the Defense Applications Research Projects Agency, Program on Carbon Electronics for RF Applications.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/999/DC1
Fig. S1

28 December 2009; accepted 1 April 2010
10.1126/science.1186489

Large Angular Jump Mechanism Observed for Hydrogen Bond Exchange in Aqueous Perchlorate Solution

Minbiao Ji,^{1,2} Michael Odelius,³ K. J. Gaffney^{1*}

The mechanism for hydrogen bond (H-bond) switching in solution has remained subject to debate despite extensive experimental and theoretical studies. We have applied polarization-selective multidimensional vibrational spectroscopy to investigate the H-bond exchange mechanism in aqueous NaClO₄ solution. The results show that a water molecule shifts its donated H-bonds between water and perchlorate acceptors by means of large, prompt angular rotation. Using a jump-exchange kinetic model, we extracted an average jump angle of $49 \pm 4^\circ$, in qualitative agreement with the jump angle observed in molecular dynamics simulations of the same aqueous NaClO₄ solution.

Hydrogen bonds (H-bonds) provide the intermolecular adhesion that dictates the unique properties of liquid water and aqueous solutions. Although H-bonds constrain the local ordering and orientation of molecules in solution, these local H-bond networks disband and reform on the picosecond time scale. This structural lability critically influences chemical and biological transformations. Our understanding of the dynamics of hydrogen bond dissociation and reformation has been transformed by the union of ultrafast vibrational spectroscopy and molecular dynamics simulations (1–14), but the detailed mechanism for H-bond switching in aqueous solution remains uncertain. Recent simulation studies of water and aqueous ionic solutions have proposed that H-bond exchange involves large angular jumps of 60° to 70° (1, 5). However, the substantial complexities inherent in simulating the structural and dynamical properties of water highlight the importance of validating this proposal experimentally.

Two-dimensional infrared (2D-IR) spectroscopy provides an excellent opportunity to study this class of ultrafast chemical exchange (15–19). We applied a variation of this technique (Fig. 1) to directly investigate the orientational jump mechanism for H-bond switching in aqueous ionic solutions. The dissolution of NaClO₄ in isotopically mixed water generates two deuterated hydroxyl (OD) stretch frequencies: OD groups donating a H-bond to another water molecule (OD_W) absorb at 2534 cm^{-1} , whereas OD groups donating a H-bond to a perchlorate anion (OD_P) absorb at 2633 cm^{-1} (Fig. 1D). This spectroscopic distinction between the OD_W and OD_P provides the opportunity to track H-bond exchange by monitoring the growth in the cross-peak intensity

in the time-dependent 2D spectra (3, 4), as shown in Fig. 1. Recent studies by Moilanen *et al.* (3) and Park *et al.* (4) have used this attribute of the water hydroxyl stretch to measure the H-bond exchange rate in aqueous NaBF₄ and NaClO₄ solutions. These experiments measured the exchange between water-water and water-anion H-bond configurations but did not directly address the hydroxyl group reorientation associated with H-bond exchange.

Two advances implemented in the present study have allowed us to extract the orientational jump angle associated with H-bond exchange. First, we have measured the laser polarization dependence of the 2D-IR spectra (20). Polarization-selective vibrational pump-probe measurements have been widely used to study the orientational dynamics of water molecules (6, 7), but these measurements cannot distinguish which water molecules have exchanged their H-bonding configuration. This can only be achieved by introducing an additional spectral dimension. Our work extends previous polarization-selective 2D-IR studies of the relative orientation of coupled vibronic states (21, 22); specifically, we address the time-dependent change in orientation of the vibrationally excited hydroxyl groups induced by chemical exchange. The second advance is a modification of the kinetic model (23) used to interpret 2D-IR spectra, so as to include vibrational transition dipole moment jump-reorientation induced by chemical exchange. Previous data analysis assumed H-bond exchange did not induce jump-reorientation (3, 4).

2D-IR spectroscopy monitors equilibrium H-bond switching dynamics on a picosecond time scale (15–19) by labeling molecules through resonant excitation at their OD stretch frequencies and then correlating these initial frequencies (ω_i) with the (potentially shifted) stretch frequencies (ω_m) associated with these same molecules after an experimentally controlled waiting time (T_W). Thus, 2D-IR can determine when a vibrationally labeled OD_W hydroxyl group converts to an OD_P configuration during T_W . The cross-peak only reflects H-bond exchange events in which

the hydroxyl group switches between the OD_W and OD_P configurations. By adding polarization selectivity, we furthermore access the orientational dynamics of water molecules that have switched between OD_W and OD_P configurations. In the polarization-selective 2D-IR measurement, the first two pulses that control the frequency labeling of OD stretches have parallel polarizations that preferentially excite transition dipole moments parallel to the excitation polarization. During the T_W waiting time, the excited molecules randomize their orientation in addition to undergoing H-bond exchange between the OD_W and OD_P configurations (Fig. 1A). If the H-bond exchange only minimally perturbs the orientation of the vibrationally excited hydroxyl group, then both the diagonal and the cross-peak intensities will exhibit similar polarization dependence. However, if the molecules exchange via large angular jumps, the cross-peak signal that results solely from OD_W-OD_P exchanged populations will show distinctly different polarization dependence from the diagonal peaks (Fig. 1, B and C).

Figure 2 shows the T_W -dependent polarization-selective 2D-IR spectra for aqueous 6M NaClO₄. The positive peaks shown in red along the diagonal ($\omega_i = \omega_m$) result from the fundamental vibrational transition ($\nu = 0 \rightarrow 1$) within each H-bond configuration. We label the peak volumes $I_{llmm}^{(ij)}$ for the diagonal ($i = j$) and the off diagonal ($i \neq j$) peaks: i and j refer to the H-bond configurations associated with ω_i and ω_m , and l and m refer to the laser polarization. The cross-peak at $(\omega_i, \omega_m) = (2534, 2633)\text{ cm}^{-1}$ results from OD_W switching to OD_P, $I_{llmm}^{(WP)}$. Because this H-bond exchange occurs at equilibrium, the exchange process from OD_P to OD_W will also generate a cross-peak $I_{llmm}^{(PW)}$ at $(\omega_i, \omega_m) = (2633, 2534)\text{ cm}^{-1}$ with $I_{llmm}^{(PW)} = I_{llmm}^{(WP)}$. Experimentally, the $\nu = 1 \rightarrow 2$ excited state absorption of OD_P obscures this cross-peak (4), so we use the $I_{llmm}^{(WP)}$ signal to characterize the jump angle. The vibrationally excited molecules that have switched between the OD_P and OD_W configurations project nearly equivalent signal intensities for perpendicular and parallel polarizations. As will be shown, this equivalence reflects the large angular jumps that lead to H-bond switching.

Figure 3 further highlights the polarization-dependent population dynamics by comparing the peak volumes for the isotropic ($I_{iso}^{(ij)} = I_{zzzz}^{(ij)} + 2I_{zzyy}^{(ij)}$) and anisotropic ($I_{aniso}^{(ij)} = I_{zzzz}^{(ij)} - I_{zzyy}^{(ij)}$) signals for a diagonal-peak ($ij = PP$) and a cross-peak ($ij = WP$). The $I_{iso}^{(PP)}$ signal reflects the population decay of the OD_P configuration, whereas $I_{aniso}^{(PP)}$ reflects population relaxation and orientational randomization of the OD_P configuration. The $I_{iso}^{(WP)}$ signal rise time reflects the rate of H-bond switching, whereas the decay results primarily from OD_P population relaxation. Unlike previous measurements of the H-bond switching time (3, 4), $I_{iso}^{(ij)}$ decouples the orientational dynamics from the H-bond configurational switching and so provides a superior measure of the exchange rate. The $I_{aniso}^{(WP)}$ signal clearly shows the very small dependence of

¹PULSE Institute for Ultrafast Energy Science, SLAC National Accelerator Laboratory, Stanford University, Stanford, CA 94305, USA. ²Department of Physics, Stanford University, Stanford, CA 94305, USA. ³Fysikum, Albnova University Center, Stockholm University, SE-106 91 Stockholm, Sweden.

*To whom correspondence should be addressed. E-mail: kgaffney@slac.stanford.edu

the cross-peak intensity on the laser polarization. The method used to extract the peak volumes can be found elsewhere (3, 23).

We modeled the polarization-selective 2D-IR spectra using diagrammatic perturbation theory and an angular jump-exchange kinetic model. This provides a framework for interpreting the time (T_W) and angle (Θ) dependent excited state population $N_i(T_W)$, orientation, and spectral diffusion dynamics (20, 23, 24). The simplest variant of the angular jump-exchange kinetic model presumes that an orientational jump through angle Θ accompanies H-bond exchange between the OD_W and OD_P configurations, whereas reorientation of hydroxyl groups occurs diffusively within a particular H-bond configuration. The model requires the angular rate of change during an angle jump to be much larger than the angular rate of change induced by orientational diffusion. As will be discussed, this requirement is consistent with our experimental and simulation results.

The angular jump-exchange kinetic model attributes the dynamics to (i) population decay with decay rates k_W and k_P , (ii) orientational diffusion with diffusion constants D_W and D_P , and (iii) chemical exchange with rate constants k_{W-P} and k_{P-W} and a H-bond exchange jump angle Θ . This model leads to ensemble-averaged populations as a function of T_W and laser polarization. For the S_{zzzz} and S_{zzyy} geometries, the polarization-dependent populations can be expressed as in Eqs. 1 and 2.

$$\begin{pmatrix} N_W(T_W) \\ N_P(T_W) \end{pmatrix}_{zzzz} = (e^{-A \cdot T_W} + \frac{4}{5} e^{-B \cdot T_W}) \times \frac{1}{3} \begin{pmatrix} N_W(0) \\ N_P(0) \end{pmatrix} \quad (1)$$

$$\begin{pmatrix} N_W(T_W) \\ N_P(T_W) \end{pmatrix}_{zzyy} = (e^{-A \cdot T_W} - \frac{2}{5} e^{-B \cdot T_W}) \times \frac{1}{3} \begin{pmatrix} N_W(0) \\ N_P(0) \end{pmatrix} \quad (2)$$

A and B correspond to matrices that contain all the kinetic coefficients, as well as Θ ,

$$A = \begin{pmatrix} k_W + k_{W-P} & -k_{P-W} \\ -k_{W-P} & k_P + k_{P-W} \end{pmatrix},$$

$$B = \begin{pmatrix} k_W + k_{W-P} + 6D_W & -\langle P_2(\cos\Theta) \rangle k_{P-W} \\ -\langle P_2(\cos\Theta) \rangle k_{W-P} & k_P + k_{P-W} + 6D_P \end{pmatrix}$$

where $P_2(\cos\Theta) = (3\cos^2\Theta - 1)/2$ and $\langle \dots \rangle$ corresponds to an ensemble average. These equations reduce to the exchange kinetic model of Kwak *et al.* (23) when $\Theta = 0^\circ$ and can be straightforwardly related to the theory of coupled vibrations developed by Golonzka and Tokmakoff (21). The A matrix has no orientational dependence and contributes solely to $I_{iso}^{(ij)}$ with dynamics determined by the excited state lifetime and the chemical exchange rate. The B matrix contributes solely to $I_{aniso}^{(ij)}$ with dynamics determined by Θ , D_W , and D_P . As shown in the supporting text, a large jump angle leads to a small $\langle P_2(\cos\Theta) \rangle$ value and the very small cross-peak anisotropy measured

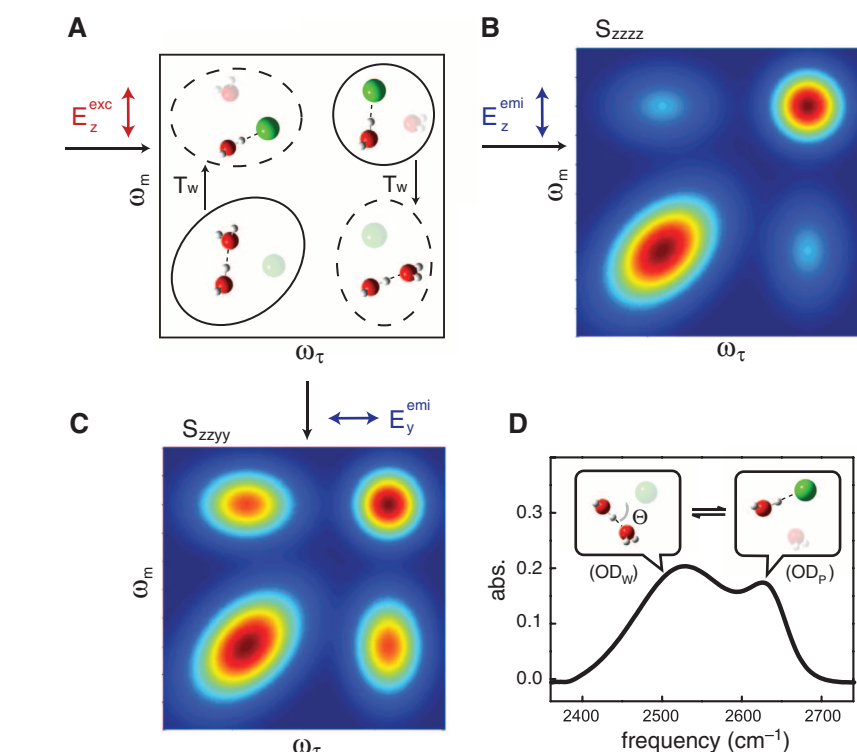


Fig. 1. Interpretation of polarization-selective 2D-IR signals. The H-bond configurations appear schematically, with the green spheres representing perchlorate ions. **(A)** Linearly polarized excitation pulses selectively label vibrational dipoles oriented parallel to the z direction, as shown in the diagonal regions of the 2D spectra. After a T_W waiting time, vibrationally labeled hydroxyl groups that have not changed H-bond configuration contribute to the diagonal component of the 2D-IR signal with minimal reorientation of the OD transition dipole. Vibrationally labeled hydroxyl groups that have undergone H-bond exchange generate cross-peak spectral intensity. If the exchange proceeds through large angular jumps, the hydroxyl groups associated with the cross-peaks will show markedly different polarization dependence than those resonating along the diagonal. This can be seen most clearly by comparing the ratio of cross-peak and diagonal-peak intensities generated with **(B)** all parallel laser polarizations (S_{zzzz} , E_z^{exc} , E_z^{emi}) and **(C)** cross-polarized laser pulses (S_{zzyy} , E_z^{exc} , E_y^{emi}). **(D)** Fourier transform IR (FTIR) absorption spectrum for 6M NaClO_4 dissolved in H_2O containing 5% monodeuterated water. The lower frequency peak at 2534 cm^{-1} corresponds to the OD stretch donating a H-bond to other water molecules (OD_W); the peak at 2633 cm^{-1} corresponds to the OD stretch donating a H-bond to a perchlorate ion (OD_P).

experimentally. We have chosen to present the simplest variant of the jump-exchange kinetic model for conceptual clarity, but we have also investigated more sophisticated models. In the supporting text, we discuss three critical aspects of the model and demonstrate that the extracted Θ value is fundamentally insensitive to the distribution of jump angles, the time evolution of the jump angle, and the detailed dynamics of the orientational randomization for hydroxyl groups that remain in a given H-bond configuration.

We used numerical response function calculations based on the above model to analyze the experimental data. The calculated anisotropic spectra appear in Fig. 2D. We used standard methods to obtain starting values for the vibrational relaxation, orientational diffusion, and frequency-frequency correlation function parameters (4, 23). The fitting of the polarization-selective 2D-IR spectra allow the extraction of the H-bond exchange rate

k_{W-P} and jump angle Θ . The analysis uses global fitting of the polarization-selective 2D-IR spectra for T_W times of 0.2, 1, 2, 3, 4, 5, and 7 ps. Fitting results give $\Theta = 49 \pm 4^\circ$, $(6D_W)^{-1} = 5 \pm 0.5 \text{ ps}$, $(6D_P)^{-1} = 4.6 \pm 0.5 \text{ ps}$, $(k_{P-W})^{-1} = 9 \pm 1 \text{ ps}$, $(k_{W-P})^{-1} = 18 \pm 2 \text{ ps}$, and a total exchange rate of $\tau_{ex} = (k_{P-W} + k_{W-P})^{-1} = 6 \pm 1 \text{ ps}$, consistent with our previous result (4). We also fit the T_W -dependent $I_{iso}^{(ij)}$ and $I_{aniso}^{(ij)}$ peak volumes, as shown in Fig. 3. These fitting results agree with the response function global fitting, within experimental error. Within the angular jump-exchange kinetic model, only large values of Θ , D_W , and D_P could lead to the very small anisotropy of the cross-peak $I_{aniso}^{(WP)}$, but the anisotropy of the diagonal peaks greatly constrains D_W and D_P . These constraints, as well as the high sensitivity of the cross-peak anisotropy to small changes in the jump angle around 49° , render the Θ value extracted from the measurements robust.

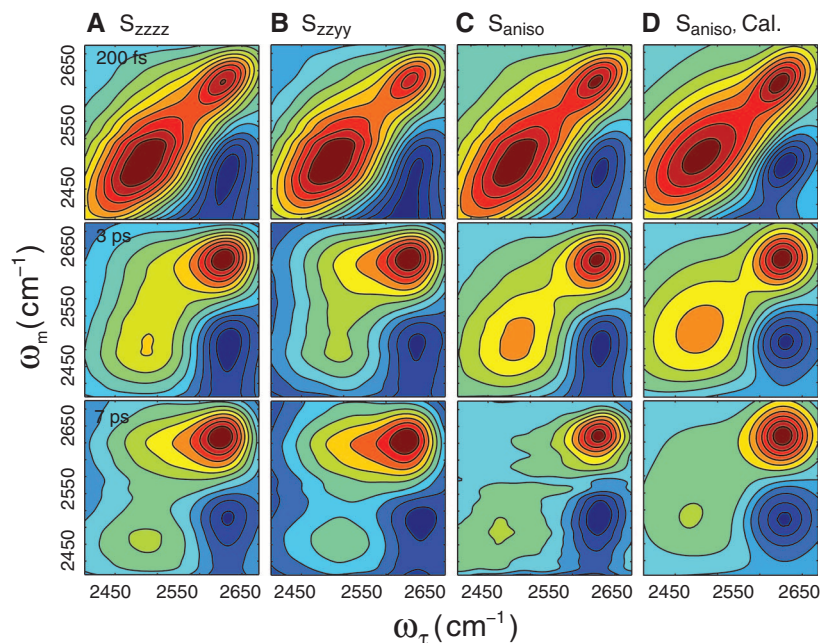


Fig. 2. Normalized polarization-selective 2D-IR spectra at $T_W = 0.2, 3$, and 7 ps. (A) Parallel polarization spectra (S_{zzzz}), (B) perpendicular polarization spectra (S_{zzyy}), and (C) anisotropic spectra, ($S_{aniso} = S_{zzzz} - S_{zzyy}$). Peak assignments can be found in the text. The cross-peaks show very little anisotropy compared with the diagonal peaks (C), clearly indicating that large-angle rotation of hydroxyl groups accompanies H-bond switching. (D) Calculated anisotropic spectra based on Eqs. 1 and 2.

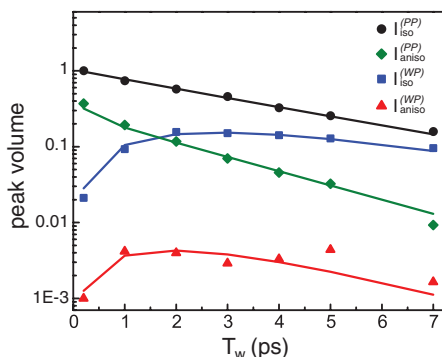


Fig. 3. Logarithmic plot of the polarization-selective peak volumes for the isotropic, $I_{ij}^{(ij)} = I_{zzzz}^{(ij)} + 2I_{zzyy}^{(ij)}$, and anisotropic, $I_{aniso}^{(ij)} = I_{zzzz}^{(ij)} - I_{zzyy}^{(ij)}$, signals for the $ij = PP$ diagonal-peak and the $ij = WP$ cross-peak. The solid lines give the kinetic model fit to the data with a H-bond exchange rate of 6 ± 1 ps and a jump angle of $49 \pm 4^\circ$.

We also performed Car-Parrinello molecule dynamics (CPMD) simulations (25) of aqueous 6M NaClO₄ to complement our experimental studies (20). The CPMD methodology differs substantially from that used previously (1, 5, 14) to study H-bond switching, yet as shown in Fig. 4, very similar angular jump dynamics emerge in the CPMD simulations of H-bond switching between OD_P and OD_W configurations (1, 5). Analysis of the mean trajectory indicates that the orientation changes on two time scales, with a 40° jump occurring with a 50-fs time constant

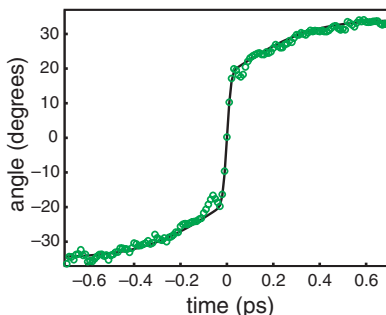


Fig. 4. CPMD simulation of the jump angle for H-bond exchange between the OD_P and the OD_W configurations for aqueous 6 M NaClO₄ solution. The data have been fit with a sum of two error functions (solid line). Results show the change in angle proceeding with two time constants, 50 fs for an initial 40° angular jump and 1 ps for a slower 27° angular rotation.

and a 27° reorientation occurring with a 1-ps time constant. By simulating the same solution as that studied experimentally, we can make a direct comparison between experiment and simulation. The results of the simulation qualitatively agree with our experimental results, validating the angular jump mechanism for H-bond switching in aqueous NaClO₄ solutions. The time constant for the fast component resembles the period of a water librational motion, consistent with the assessment that the orientational jump reflects a primarily concerted rotational motion. The 1-ps

process occurs too slowly to be viewed as concerted, but our experiment cannot accurately measure the detailed time evolution of the angular jump because the cross-peak intensity grows in with $\tau_{ex} = 6$ ps. Nonetheless, for $T_W \leq 2$ ps, the cross-peak shows a very small anisotropy (fig. S8), which has been accurately modeled with an impulsive angular jump and conforms to the predominantly subpicosecond angular dynamics observed in the simulation. Whether the lack of quantitative agreement between experiment and simulation reflects limitations in the CPMD simulation or the modeling of the experimental data awaits further investigation.

References and Notes

1. D. Laage, J. T. Hynes, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 11167 (2007).
2. C. J. Fecko, J. D. Eaves, J. J. Loparo, A. Tokmakoff, P. L. Geissler, *Science* **301**, 1698 (2003).
3. D. E. Moilanen, D. Wong, D. E. Rosenfeld, E. E. Fenn, M. D. Fayer, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 375 (2009).
4. S. Park, M. Odelius, K. J. Gaffney, *J. Phys. Chem. B* **113**, 7825 (2009).
5. D. Laage, J. T. Hynes, *Science* **311**, 832 (2006).
6. Y. L. A. Rezus, H. J. Bakker, *J. Chem. Phys.* **123**, 114502 (2005).
7. H. J. Bakker, *Chem. Rev.* **108**, 1456 (2008).
8. C. P. Lawrence, J. L. Skinner, *J. Chem. Phys.* **118**, 264 (2003).
9. J. B. Asbury *et al.*, *J. Phys. Chem. A* **108**, 1107 (2004).
10. J. D. Eaves *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13019 (2005).
11. M. L. Cowan *et al.*, *Nature* **434**, 199 (2005).
12. B. Nigro, S. Re, D. Laage, R. Rey, J. T. Hynes, *J. Phys. Chem. A* **110**, 11237 (2006).
13. A. Luzar, D. Chandler, *Phys. Rev. Lett.* **76**, 928 (1996).
14. R. Ludwig, *Chem. Phys. Chem.* **8**, 44 (2007).
15. J. F. Cahoon, K. R. Sawyer, J. P. Schlegel, C. B. Harris, *Science* **319**, 1820 (2008).
16. J. Zheng *et al.*, *Science* **309**, 1338 (2005).
17. J. Zheng, K. Kwak, J. Xie, M. D. Fayer, *Science* **313**, 1951 (2006).
18. S. Woutersen, Y. Mu, G. Stock, P. Hamm, *Chem. Phys.* **266**, 137 (2001).
19. Y. S. Kim, R. M. Hochstrasser, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11185 (2005).
20. Methods are detailed in supporting online material at Science Online.
21. O. Golonzka, A. Tokmakoff, *J. Chem. Phys.* **115**, 297 (2001).
22. M. T. Zanni, N. H. Ge, Y. S. Kim, R. M. Hochstrasser, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 11265 (2001).
23. K. Kwak, J. Zheng, H. Cang, M. D. Fayer, *J. Phys. Chem. B* **110**, 19998 (2006).
24. A. Tokmakoff, *J. Chem. Phys.* **105**, 1 (1996).
25. R. Car, M. Parrinello, *Phys. Rev. Lett.* **55**, 2471 (1985).
26. M.J. and K.J.G. were supported through the PULSE Institute at the SLAC National Accelerator Laboratory by the U.S. Department of Energy Office of Basic Energy Sciences. M.O. acknowledges support from the Carl Tryggers and Magnus Bergwall foundations and the Swedish Research Council, and computer time allocations at the Swedish National Supercomputer Center and Center for Parallel Computing (PDC).

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/1003/DC1
Materials and Methods
Figs. S1 to S11
Table S1
References

29 January 2010; accepted 16 March 2010
10.1126/science.1187707

Cooperativity in Ion Hydration

K. J. Tielrooij, N. Garcia-Araez, M. Bonn, H. J. Bakker*

Despite prolonged scientific efforts to unravel the effects of ions on the structure and dynamics of water, many open questions remain, in particular concerning the spatial extent of this effect (i.e., the number of water molecules affected) and the origin of ion-specific effects. A combined terahertz and femtosecond infrared spectroscopic study of water dynamics around different ions (specifically magnesium, lithium, sodium, and cesium cations, as well as sulfate, chloride, iodide, and perchlorate anions) reveals that the effect of ions and counterions on water can be strongly interdependent and nonadditive, and in certain cases extends well beyond the first solvation shell of water molecules directly surrounding the ion.

The properties of solutions of ions in water are of relevance for a wide range of systems, including biological environments (1) and atmospheric aerosols (2). Even for simple binary solutions, the effect of ions on the structure and dynamics of water has been the subject of ongoing debate (3–5). Key questions concerning ion effects on water pertain to the number of water molecules affected and how the degrees of freedom of these water molecules are influenced.

During the past decade, a variety of measurement techniques have provided evidence that ions primarily have an effect on the structure and dynamics of the first solvation shell of water molecules directly surrounding the ion. This evidence consists of structural measurements of ion hydration using neutron and x-ray diffraction (6, 7), x-ray absorption spectroscopy (8), and infrared and Raman spectroscopy (9). Information on the effects of ions on water dynamics has been mainly obtained from femtosecond time-resolved infrared (fs-IR) vibrational spectroscopy (10–12) and optical Kerr-effect spectroscopy (13). These reports support the notion that the effect of ions on water is largely limited to the first solvation shell. A different dynamical technique, dielectric relaxation (DR) spectroscopy, also showed that for many different cations and anions, the effect is limited to the first solvation shell (14–17). However, for certain ion combinations, an effect beyond the first solvation shell was observed (18–20). It is currently unclear what factors determine the degree of hydration and what constitutes the molecular-level structure and dynamics; a unifying molecular picture of ion hydration is lacking.

Here, we studied the effect of ions on water by means of fs-IR spectroscopy and terahertz DR spectroscopy. It turns out that these techniques are uniquely complementary, in that they are sensitive to water reorientation dynamics along different molecular axes of water. Moreover, the ability to independently resolve water reorientation along different directions helps to uncover previously unappreciated cooperativity between

hydrated cations and anions. We studied dissolved salts containing various combinations of ions that have different charge densities and water affinities. For specific combinations of cations and anions, we observed dynamic hydration effects that extend well beyond the first structural solvation shell.

We first present the results of DR spectroscopy of five different dissolved salts (21). These measurements were performed by characterizing in time the propagation of a terahertz (THz) pulse with a duration of ~ 1 ps through the salt solution. In the inset of Fig. 1A, we show THz pulses as transmitted through solutions of Cs_2SO_4 (0.5 mol/kg), MgSO_4 (0.5 mol/kg), and a reference solution of CsCl (16). Generally, THz pulses are delayed as a result of refraction and experience a decrease in amplitude as a result of absorption. In water, a marked frequency dependence of refraction and absorption arises when the dipoles of water molecules fail to keep up reorienting with an externally applied oscillating electric field. This is the DR process, which leads to a large absorption peak at 20 GHz and a smaller one around 0.6 THz, for pure water molecules at room temperature. These peaks are attributed to the collective reorientation of water molecules (Debye relaxation time $\tau_D \approx 8$ ps) and the reorientation of under-coordinated water molecules (relaxation time $\tau_2 \approx$

250 fs), respectively (22, 23). In Fig. 1A, we show the imaginary part of the extracted dielectric function ϵ , which is associated with the absorption of electromagnetic radiation by water molecules. As a result of the interaction with solvated ions, the reorientation of water molecules around ions slows down, shifting the absorption peak to lower frequencies and thereby reducing the absorption of radiation at THz frequencies (depolarization).

It is typically found that the dynamics of water molecules in salt solutions are nonuniform and show two or more time scales. Previous dielectric relaxation studies found that a certain fraction of water molecules displays very slow reorientation dynamics, whereas for the remaining water molecules the orientational dynamics are similar to those of bulk liquid water (14–20). Also, computer simulations found that a fraction of water remains unaffected by the presence of the ions (24, 25). In analyzing the THz DR data, we follow the literature by identifying two sub-ensembles of water molecules in ionic solution: those whose dynamics are predominantly unaffected by the presence of ions, and those whose dynamics are slowed down. Both sub-ensembles are characterized by a distribution of reorientation time scales.

In Fig. 1B, we show the concentration-dependent depolarization and corresponding slow water fraction. The hydration number N_p is defined as the number of moles of slow water dipoles (p) per mole of dissolved salt and is directly proportional to the slopes of the lines in Fig. 1B (14–20). The hydration number N_p is a dynamical property and is therefore distinct from the structural concept of solvation shells. Solvation shells are often defined in terms of the distance distribution of the water molecules from the center of the ion, but as such do not present information on the dynamics of the water molecules. It is typically found that ions with a larger charge density (small, multivalent ions) affect the dy-

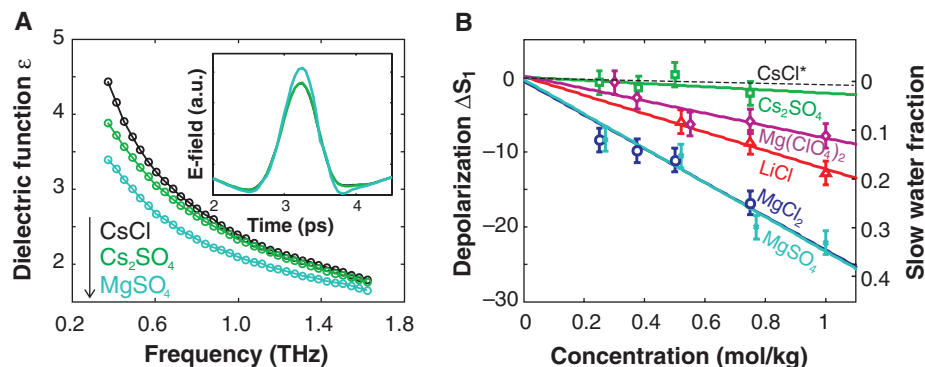


Fig. 1. Results of DR spectroscopy. **(A)** The imaginary part of the dielectric function ϵ'' for solutions of CsCl , Cs_2SO_4 , and MgSO_4 (all 0.5 mol/kg) and the corresponding transmitted THz pulses (inset). **(B)** Depolarization [corrected for kinetic depolarization due to conductivity (21)] and fraction of slow water as a function of concentration for the investigated salt solutions. The fraction of slow water is equal to the depolarization divided by the difference between the dielectric response of pure water at zero and infinite frequency, which equals 74 at 298 K. The lines are linear fits to the depolarization values (or slow fractions) and serve to distinguish the studied salts. Error bars represent the 95% confidence interval and are derived through error propagation of the experimental uncertainty in the dielectric function. *Data from (16).

FOM Institute for Atomic and Molecular Physics (AMOLF), Science Park 104, 1098 XG Amsterdam, Netherlands.

*To whom correspondence should be addressed. E-mail: bakker@amolf.nl

namics of a larger number of water molecules (i.e., have a higher hydration number) than ions with a lower charge density (large, monovalent ions) (6, 7, 14–16, 18–20). This is caused by the larger local electric field around small, multivalent ions, which affects the orientation of a larger number of surrounding water molecules.

Our results for MgCl_2 , LiCl , and CsCl are in good agreement with this general rule. For $\text{Mg}(\text{ClO}_4)_2$ we find $N_p = 6$; because ClO_4^- is a very weakly hydrated anion, the observed slowly reorienting water molecules are likely located in the first geometrically surrounding solvation shell of the Mg^{2+} ion. In contrast, for Cs_2SO_4 , N_p is only 1. Apparently, the water molecules in the solvation shell of Cs^+ show reorientational

dynamics similar to those of bulk liquid water (16), probably because the positive charge of the Cs^+ ion is distributed over a large volume: The slowly reorienting water can be attributed to a water molecule located within the solvation shell of strongly hydrated SO_4^{2-} , presumably forming hydrogen bonds with both OH groups to two oxygen atoms of the SO_4^{2-} ion. The low value of N_p found for Cs_2SO_4 indicates that the effect of anions on water reorientation is either negligible or not measurable by DR measurements. Similarly, a previous GHz DR measurement of the anions Br^- , I^- , NO_3^- , ClO_4^- , and SCN^- found that the impact of these anions on water dynamics is remarkably small and remarkably similar, despite the different water affinities of these ions (17).

We used fs-IR measurements (21) to complement our DR measurements. The fs-IR technique allows direct study of the reorientational dynamics of individual water molecules with high temporal resolution (~ 150 fs). In these experiments, we excite the OD-stretch vibration of a subset of HDO molecules in H_2O (4% D_2O in H_2O). Molecules with their OD group preferentially aligned along the polarization axis of the excitation (pump) pulse are most efficiently tagged. By using a second laser (probe) pulse to interrogate the number of tagged molecules parallel and perpendicular to the excitation axis, the rotation of tagged molecules can be followed in time. The anisotropy $R(t)$ [corrected for heating due to the excitation according to (26)] as a function of pump-probe delay time t reflects the orientational dynamics of the probed water molecules. For bulk water, the decay time constant of $R(t)$ is directly related to the Debye relaxation time τ_D , as measured by DR spectroscopy (27). Figure 2, A to C, shows the anisotropy decay for the dissolved salts $\text{Mg}(\text{ClO}_4)_2$, Cs_2SO_4 , and MgSO_4 , each for concentrations ranging from 0 to 2 mol/kg. A remarkable conclusion can be drawn from these three measurements: MgSO_4 shows a very large slow reorientation component, whereas Mg^{2+} and SO_4^{2-} individually, in combination with other ions (ClO_4^- and Cs^+ , respectively), do not. This shows that the effect of ions on water dynamics can be nonadditive (as discussed below).

Before discussing the non-additivity, we focus our attention on the observation that there is an apparent discrepancy between the DR measurements and the fs-IR measurements. For hydrated cations, DR and fs-IR spectroscopies give different results: Mg^{2+} and Li^+ show large immobilized fractions when measured with DR, but the fs-IR measurements of $\text{Mg}(\text{ClO}_4)_2$ and LiI show complete reorientation, with a negligible slow fraction (Fig. 2D). These fractions have been obtained using a bimodal model, analogous to the DR measurements: The slow time constant and the associated fraction of hydration water are obtained from a double-exponential fit to the anisotropy decay, in which the fast time constant equals the reorientation time constant of 2.6 ps for bulk water. This fast time constant is determined in an independent measurement performed in the absence of salt. The slow component represents a weighted average of water molecules that have in common that they reorient much more slowly than the water molecules in bulk liquid water. Note that the exchange of water molecules between the dynamic hydration shells and the bulk typically takes place on a time scale of tens to hundreds of picoseconds (28). This exchange time scale is much longer than the reorientation time of ~ 2.6 ps of bulk liquid water. Hence, the reorientation of the water molecules outside the dynamic hydration shells is well separated from the reorientation dynamics of the water molecules in the dynamic hydration shells. In analogy to the DR measurements, the slow water fraction as measured with fs-IR spectroscopy can be translated

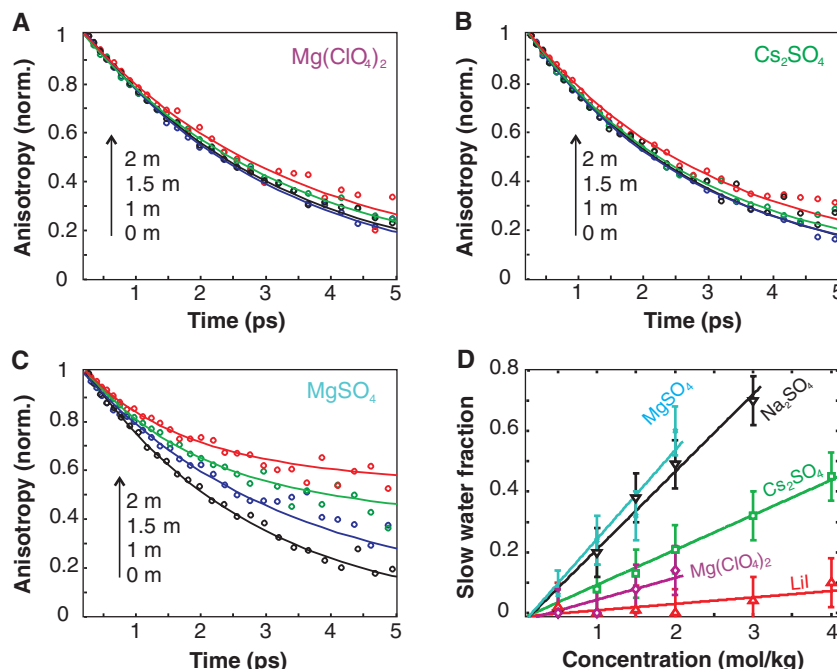
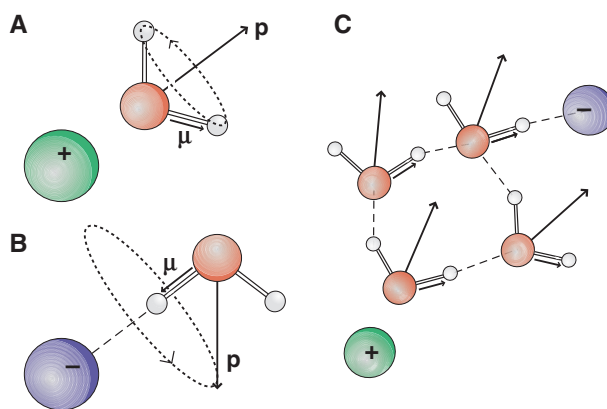


Fig. 2. Results of fs-IR spectroscopy. (A to C) The normalized decay of the anisotropy $R(t)$ for $\text{Mg}(\text{ClO}_4)_2$ (A), Cs_2SO_4 (B), and MgSO_4 (C) at concentrations of 0, 1, 1.5, and 2 mol/kg. We fit the anisotropy decay of all different salt solutions using double exponentials with floating amplitudes and fixed time scales: a time scale of 2.6 ps, as in bulk water (26), and a slow water time scale of 10 ps. (D) The fraction of slow water relative to bulk-like water. The lines are linear fits and serve as guides to the eye to distinguish the studied salts. The error bars are based on at least four measurement runs and represent the 95% confidence interval.

Fig. 3. Semi-rigid hydration and cooperativity. (A and B) A water molecule in the solvation shell of a cation (A) and an anion (B). Dielectric relaxation measurements probe the reorientation of the permanent dipole vector $\mathbf{p}$. Femtosecond infrared spectroscopy is sensitive to the reorientation of the OD-stretch transition dipole moment $\boldsymbol{\mu}$. The dotted arrows indicate reorientation in a cone, in the case of semi-rigid hydration. (C) Proposed geometry, in which the water dynamics are locked in two directions because of the cooperative interaction with the cation and the anion.



into a hydration number N_{μ} . This corresponds to the number of moles of slowly reorienting OH groups (with transition dipole moment μ) per mole of dissolved salt (keeping in mind that there are two OH groups per water molecule).

The differences between the results of DR and fs-IR spectroscopies, in terms of their apparent sensitivity toward the dynamics of water hydrating cations and anions, can be understood by noting the different vectors that the two measurement techniques probe: the permanent dipole moment of water molecules $\mathbf{p}$ in the case of DR spectroscopy, and the OD-stretch transition dipole moment μ in the case of fs-IR spectroscopy (Fig. 3). The local electric field around the ions causes the dipole vector $\mathbf{p}$ of water molecules in the solvation shell of a cation to point radially away from the cation, whereas for an anion, one of the OH groups of a hydrogen-bonded water mole-

cule linearly points toward the anion (9, 29). From Fig. 3, it is clear that there is rotational motion of water molecules in the cationic solvation shell, which does not lead to reorientation of the vector $\mathbf{p}$, but does result in randomization of the transition dipole vector μ . For the case of anions, the reverse effect occurs: For water molecules in the anionic solvation shell, the motion of $\mathbf{p}$ is unrestricted within a cone with fixed axis μ , where μ is the OD bond that is hydrogen-bonded to the anion. Reorientation in a cone with a semi-cone angle between μ and $\mathbf{p}$ of $\alpha \approx 52^\circ$ (half the HOD-bond angle) leads to a complete randomization of the vector whose motion is unrestricted within the cone (27). This explains the insensitivity of DR spectroscopy toward anionic hydration and of fs-IR spectroscopy toward cationic hydration. For both cations and anions, these observations lead to a molecular picture of semi-rigid hydration (i.e., water molecules in ionic solvation shells that reorient in a propeller-like manner), giving rise to partial reorientation along a distinct axis. The molecular picture of semi-rigid hydration explains the ion effect on water molecules directly surrounding the ion. This picture holds for salts for which one of the counterions is weakly hydrated. However, when both ions are strongly hydrated, the effect on water dynamics can be much stronger and non-additive. The THz DR data show that $\text{Mg}(\text{ClO}_4)_2$ has a hydration number $N_p = 6$ and that Cs_2SO_4 has a hydration number $N_p = 1$; these values are associated with water molecules directly adjacent to the Mg^{2+} ion and a water molecule hydrating the SO_4^{2-} ion, respectively. For MgSO_4 , $N_p = 18$, which is much larger than the sum of the hydration numbers of $\text{Mg}(\text{ClO}_4)_2$ and Cs_2SO_4 (Fig. 1). This means that the dynamics of a large number of water molecules are affected because of a cooperative effect of the cation and the anion. The size of most ions allows them to be structurally surrounded by four to six water molecules. Hence, a value of $N_p > 6$ and $N_{\mu} > 12$ implies that the effect of the ion on the orientational dynamics of water extends well beyond the first structurally surrounding shell of water molecules.

The same cooperativity is observed in the fs-IR measurements (Fig. 2), where MgSO_4 has a much larger fraction of slowly reorienting water molecules (corresponding to a hydration number $N_{\mu} = 32$) than $\text{Mg}(\text{ClO}_4)_2$ and Cs_2SO_4 (with hydration numbers N_{μ} of 4 and 9, respectively). For MgSO_4 , there are about twice as many slowly rotating OH groups (N_{μ}) as slowly rotating dipoles (N_p), which indicates that the same collection of slow water molecules is observed by fs-IR and THz DR spectroscopies. Even the combination of the moderately strongly hydrated cation Na^+ with the strong anion SO_4^{2-} is observed to affect the dynamics of a large number of water molecules ($N_{\mu} = 24$; Fig. 2D). Thus, the effects of ions and counterions can be strongly interdependent and nonadditive. The key parameter determining how strongly ions affect water dynamics is thus the combination of the solvated cation and anion.

It is clear that the effect of MgSO_4 and Na_2SO_4 on water extends well beyond the first solvation shell of the ions and that the ions show strong cooperativity in affecting the dynamics of water molecules. Note that this effect is not related to ion pairing. Previous GHz DR studies by Buchner *et al.* showed the presence of a certain amount of ion pairs for solutions of MgSO_4 and Na_2SO_4 (18, 20). Ion pairs lead to additional resonances at very low frequencies (<5 GHz), located well outside our THz DR measurement window (0.4 to 1.2 THz). For our fs-IR measurements we can also neglect the direct contribution of ion pairs to the anisotropy data, because this technique excites and probes specifically the hydroxyl vibrations of water molecules. Hence, the slowing of the anisotropy decay for MgSO_4 and Na_2SO_4 corresponds to the slow reorientation of water molecules, not the slow reorientation of ion pairs. A remaining question is whether the observed slow water molecules could be water that hydrates ion pairs. This is in fact very unlikely because the amount of ion pairing is generally small—less than 10% for MgSO_4 , even at high salt concentrations (18)—whereas the observed cooperative effect leads to a slowing down of a large fraction of the water (up to 70% of all water molecules in the solution; see Fig. 2D). Hence, the slow water fraction is associated with hydration of nonpaired Mg^{2+} and SO_4^{2-} .

The cooperativity in ion hydration can be explained by the fact that the cation and anion lock different degrees of freedom of the water molecules—that is, the direction of the bisectrix ($\mathbf{p}$) and the direction of OH (μ), respectively. The nearby presence of both ions can thus lead to a locking in both directions of the hydrogen-bond structure of several intervening water layers, giving rise to the observation by DR and fs-IR of slowed water molecules well beyond the first solvation shell. This cooperativity is schematically illustrated in Fig. 3C. We expect the solvation structures to be quite directional between the ions; if an ion forms ~ 4 of these structures with surrounding counterions, the value of $N_p = 18$ implies that each of these structures consists, on average, of four or five water molecules. This interpretation also means that for solutions such as MgSO_4 and Na_2SO_4 , the slowly reorienting water molecules are not arranged in a spherically symmetric way around the ions.

The reorientation of water molecules in the rigid, locked hydrogen-bond structure occurring for MgSO_4 can be expected to show a temperature dependence different from that of pure liquid water. We measured the temperature dependence of the anisotropy decay for a MgSO_4 solution (1.5 mol/kg) over an interval of about 50°C (Fig. 4A). We also compared the temperature dependence to that of a Cs_2SO_4 solution (4 mol/kg) (Fig. 4B) with the same hydration level (Fig. 2D) but no cooperativity. At room temperature, the anisotropy for MgSO_4 decays more slowly than for Cs_2SO_4 , but with increasing temperature the situation reverses. To quantify

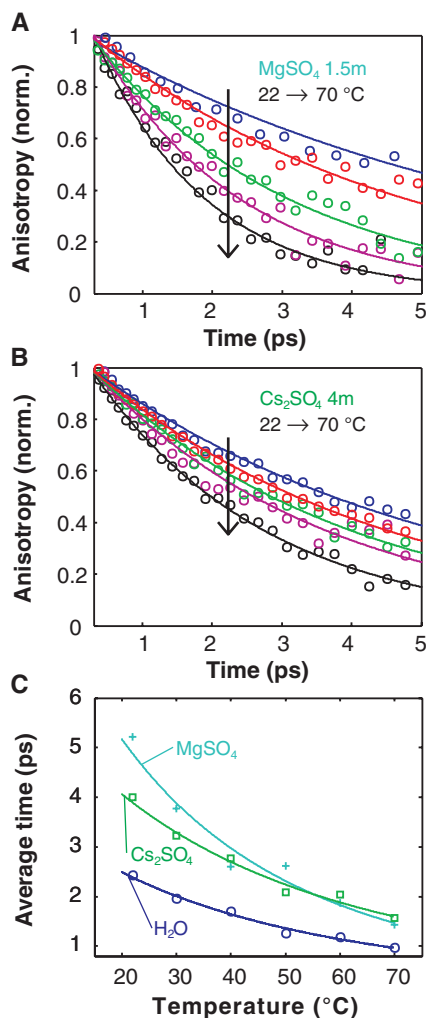


Fig. 4. Temperature dependence of the reorientation. (A and B) Temperature-dependent anisotropy decay data for MgSO_4 (A) and Cs_2SO_4 (B) with fits as explained in the text. (C) Average reorientation times of solutions of MgSO_4 (1.5 mol/kg), Cs_2SO_4 (4 mol/kg), and neat H_2O [from (27)] as a function of temperature.

the results, we fit the anisotropy data at different temperatures with a single averaged time scale for all water molecules in the system. This means that a change in this time scale represents both the change in time scales and change in the relative fractions of bulk-like and slow water molecules. Figure 4C shows a much stronger temperature dependence of this average time scale for a solution of MgSO_4 (1.5 mol/kg) than for a solution of Cs_2SO_4 (4 mol/kg).

The difference in temperature dependence of the reorientation of MgSO_4 and Cs_2SO_4 solutions indicates that the reorientation within the hydration structures involves a different mechanism. For pure water, molecular dynamics simulations indicated that the reorientation of water follows a concerted mechanism and that the rate-limiting step for reorientation of a water molecule is the motion of a second water molecule in and out of the solvation shell of the first (30, 31). For the Cs_2SO_4 solution, the hydration number N_{μ} has a value of 9, which is likely associated with the OH groups of water molecules that are hydrogen-bonded to the SO_4^{2-} ion. In view of the small value of N_{μ} , these water molecules are surrounded by water molecules that show bulk-like dynamics. Hence, although the reorientation of the water molecules hydrating the SO_4^{2-} is slow, the temperature dependence of this reorientation is similar to that of pure liquid water, because the reorientation is governed by hydrogen-bond interactions to water molecules that show bulk-like behavior. Correspondingly, the temperature dependence of the reorientation time of Cs_2SO_4 is similar to that of bulk water. In contrast, for MgSO_4 the solvation structures are large, as expressed by the large values of the hydration numbers $N_p = 18$ and $N_{\mu} = 32$. Hence, the reorientation of a water molecule in the solvation structure relies on the motions of water molecules that are contained in the same solvation structure. These motions are substantially slowed down, and reorientation thus involves a collective reorganization of the extended solvation structure, which explains the difference in temperature dependence with a solution of Cs_2SO_4 and pure liquid water.

Our results show that the hydration structure of a strongly hydrated ion depends critically on the nature of its counterion. If the counterion is weakly hydrated, the strongly hydrated ion is surrounded by a semi-rigid solvation shell, where reorientation is restricted only in a certain direction but is still allowed in other directions. However, if strongly hydrated cations and anions are combined, the dynamics of water molecules well beyond the first solvation layer are affected. In this case, the hydrogen-bond network is locked in multiple directions. These findings show that the effect of ions on water dynamics can be strongly interdependent and nonadditive.

References and Notes

- W. Kunz, J. Henle, B. W. Ninham, *Curr. Opin. Colloid Interface Sci.* **9**, 19 (2004).
- P. Jungwirth, D. J. Tobias, *Chem. Rev.* **106**, 1259 (2006).
- Y. Marcus, *Chem. Rev.* **109**, 1346 (2009).
- D. J. Tobias, J. C. Hemminger, *Science* **319**, 1197 (2008).
- F. Franks, Ed., *Water: A Comprehensive Treatise* (Plenum, New York, 1973).
- R. Mancinelli, A. Botti, F. Bruni, M. A. Ricci, A. K. Soper, *J. Phys. Chem. B* **111**, 13570 (2007).
- K. D. Collins, G. W. Neilson, J. E. Enderby, *Biophys. Chem.* **128**, 95 (2007).
- C. D. Cappa, J. D. Smith, B. M. Messer, R. C. Cohen, R. J. Saykally, *J. Phys. Chem. B* **110**, 5301 (2006).
- J. D. Smith, R. J. Saykally, P. L. Geissler, *J. Am. Chem. Soc.* **129**, 13847 (2007).
- A. W. Omta, M. F. Kropman, S. Woutersen, H. J. Bakker, *Science* **301**, 347 (2003).
- S. Park, M. D. Fayer, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 16731 (2007).
- D. E. Moilanen, D. Wong, D. E. Rosenfeld, E. E. Fenn, M. D. Fayer, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 375 (2009).
- D. A. Turtin, J. Hunger, G. Hefter, R. Buchner, K. Wynne, *J. Chem. Phys.* **128**, 161102 (2008).
- U. Kaatz, *J. Solution Chem.* **26**, 1049 (1997).
- R. Buchner, G. T. Hefter, P. M. May, *J. Phys. Chem. A* **103**, 1 (1999).
- T. Chen, G. Hefter, R. Buchner, *J. Phys. Chem. A* **107**, 4025 (2003).
- W. Wachter, W. Kunz, R. Buchner, G. Hefter, *J. Phys. Chem. A* **109**, 8675 (2005).
- R. Buchner, T. Chen, G. Hefter, *J. Phys. Chem. B* **108**, 2365 (2004).
- W. Wachter, S. Fernandez, R. Buchner, G. Hefter, *J. Phys. Chem. B* **111**, 9010 (2007).
- R. Buchner, S. G. Capewell, G. Hefter, P. M. May, *J. Phys. Chem. B* **103**, 1185 (1999).
- See supporting material on Science Online.
- J. T. Kindt, C. A. Schmuttenmaer, *J. Phys. Chem.* **100**, 10373 (1996).
- C. Rønne et al., *J. Chem. Phys.* **107**, 5319 (1997).
- D. Laage, J. T. Hynes, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 11167 (2007).
- Y. S. Lin, B. M. Auer, J. L. Skinner, *J. Chem. Phys.* **131**, 144511 (2009).
- Y. L. A. Rezus, H. J. Bakker, *J. Chem. Phys.* **123**, 114502 (2005).
- K. J. Tielrooij, C. Petersen, Y. L. A. Rezus, H. J. Bakker, *Chem. Phys. Lett.* **471**, 71 (2009).
- S. Koneshan, J. C. Rasaiah, R. M. Lynden-Bell, S. H. Lee, *J. Phys. Chem. B* **102**, 4193 (1998).
- H. Ohtaki, T. Radnai, *Chem. Rev.* **93**, 1157 (1993).
- D. Laage, J. T. Hynes, *Science* **311**, 832 (2006); published online 26 January 2006 (10.1126/science.1122154).
- D. Laage, J. T. Hynes, *J. Phys. Chem. B* **112**, 14230 (2008).
- This work is part of the research program of the Stichting voor Fundamenteel Onderzoek der Materie (FOM), which is financially supported by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO). We thank H. Schoenmaker for technical support, R. L. A. Timmer and E. H. G. Backus for useful discussions, and D. Frenkel and E. W. Meijer for helpful comments on the manuscript.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/1006/DC1
Materials and Methods
Figs. S1 to S3
References

19 October 2009; accepted 11 March 2010
10.1126/science.1183512

Self-Assembly of Janus Dendrimers into Uniform Dendrimerosomes and Other Complex Architectures

Virgil Percec,^{1*} Daniela A. Wilson,¹ Pawaret Leowanawat,¹ Christopher J. Wilson,¹ Andrew D. Hughes,¹ Mark S. Kaucher,¹ Daniel A. Hammer,^{2,3} Dalia H. Levine,³ Anthony J. Kim,³ Frank S. Bates,⁴ Kevin P. Davis,⁴ Timothy P. Lodge,^{4,5} Michael L. Klein,⁶ Russell H. DeVane,⁶ Emad Aqad,¹ Brad M. Rosen,¹† Andreea O. Argintaru,¹ Monika J. Sienkowska,¹ Kari Rissanen,⁷ Sami Nummelin,^{7,8} Jarmo Ropponen⁷

Self-assembled nanostructures obtained from natural and synthetic amphiphiles serve as mimics of biological membranes and enable the delivery of drugs, proteins, genes, and imaging agents. Yet the precise molecular arrangements demanded by these functions are difficult to achieve. Libraries of amphiphilic Janus dendrimers, prepared by facile coupling of tailored hydrophilic and hydrophobic branched segments, have been screened by cryogenic transmission electron microscopy, revealing a rich palette of morphologies in water, including vesicles, denoted dendrimerosomes, cubosomes, disks, tubular vesicles, and helical ribbons. Dendrimerosomes marry the stability and mechanical strength obtainable from polymersomes with the biological function of stabilized phospholipid liposomes, plus superior uniformity of size, ease of formation, and chemical functionalization. This modular synthesis strategy provides access to systematic tuning of molecular structure and of self-assembled architecture.

Biological membranes are complex systems assembled from phospholipids and stabilized by cholesterol, proteins, and carbohydrates (1). They are equipped with nanoscale machinery that includes protein channels to mediate and control electron and proton transfer between the cell and its environment. Liposomes (2), vesicles assembled from natural or synthetic amphiphiles, can mimic biological membranes

(1, 3–6), probe cell machinery (3, 6), and can be configured into biomimetic materials for nanomedicine and other applications (7–9). Designing vesicles endowed with specific functional features (including tunable mechanical strength, targeted size, uniform size distributions, and tailored transport properties) presents a formidable challenge. Both synthetic (10–16) and natural (16, 17) vesicles are typically polydisperse and unstable

¹Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104–6323, USA. ²Department of Bioengineering and Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA. ³Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104–6323, USA. ⁴Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455, USA. ⁵Department of Chemistry, University of Minnesota, Minneapolis, MN 55455–0431, USA. ⁶Institute for Computational Molecular Science, Temple University, Philadelphia, PA 19122, USA. ⁷Nanoscience Center, Department of Chemistry, University of Jyväskylä, 40014 JYU, Finland. ⁸Molecular Materials, Department of Applied Physics, School of Science and Technology, Aalto University, FI-02150, Espoo, Finland.

*To whom correspondence should be addressed. E-mail: percec@sas.upenn.edu

†Present address: DuPont Central Research and Development, Wilmington, DE 19880, USA.

over time, necessitating tedious fractionation (14–17) and stabilization (8, 9, 18–21), because specific applications are decided by these factors. Here we report the preparation of a broad class of synthetic amphiphiles known as Janus dendrimers (22) and demonstrate facile self-assembly in water of stable bilayer vesicles referred to as dendrimersomes. Most of these dispersions contain populations of bilayer capsules remarkably uniform in size. We document superior mechanical properties and impermeability to encapsulated compounds. Dendrimersomes can also incorporate pore-forming proteins, coassemble with structure-directing phospholipids and block copolymers, and offer a molecular periphery suitable for further chemical functionalization. This report provides the first description of the

preparation, structure, and properties of dendrimersomes (and other select complex dispersions) discovered by screening extensive libraries of Janus dendrimers prepared with exacting chemical fidelity.

Dendrimers are technologically advanced synthetic compounds with monodisperse and precisely branched molecular architectures (22–24). Many factors control the ultimate properties and applications of these fascinating materials, including the number of primary branches that emanate from a common linking point, the density of radial branches, and the distribution of chemical moieties located inside the molecular labyrinth or at the periphery of the molecule. A Janus dendrimer is formed by linking two chemically distinct dendritic building blocks, thereby breaking the rough-

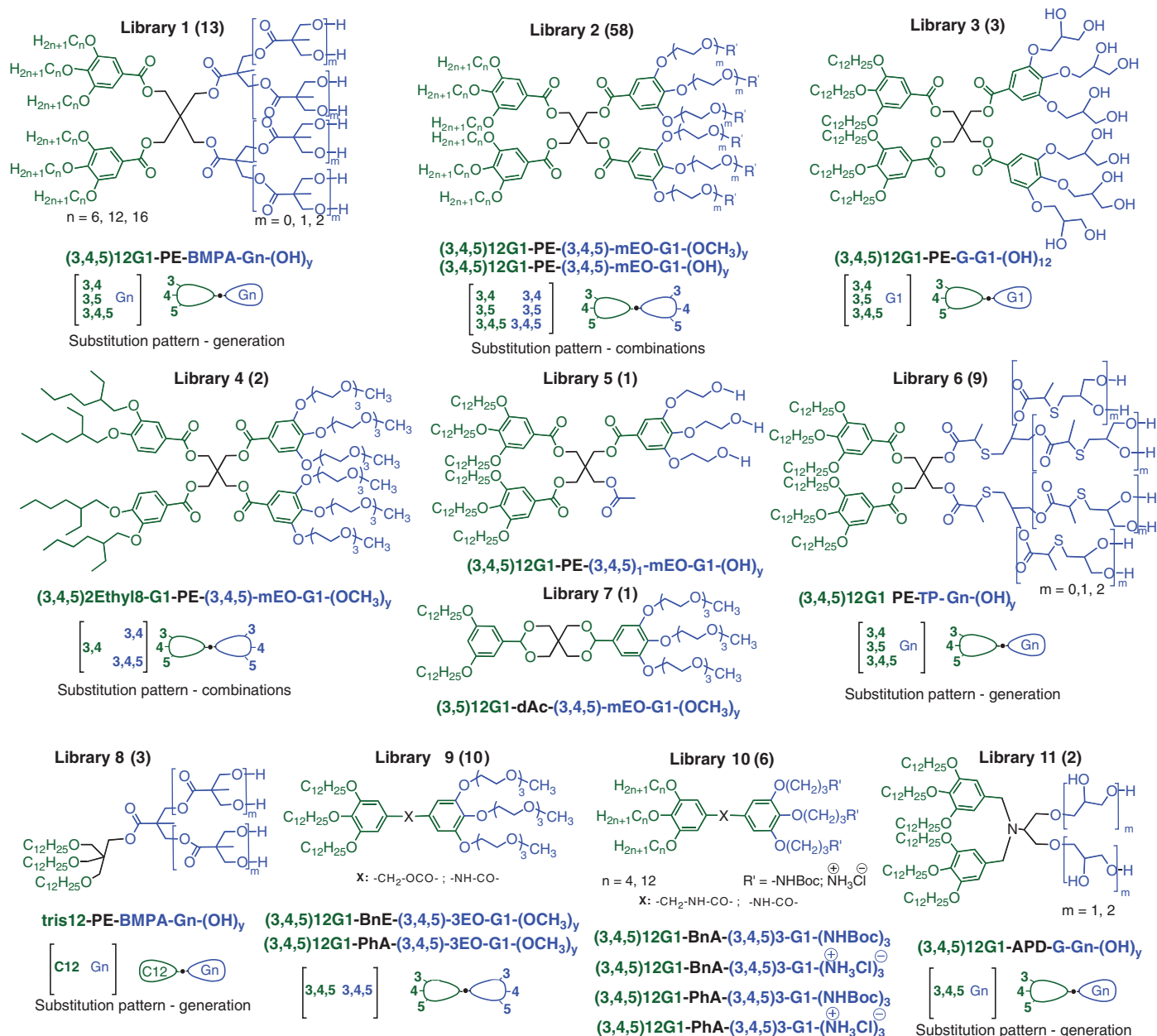


Fig. 1. Libraries of amphiphilic Janus dendrimers.

ly spherical symmetry that characterizes most dendrimers (22–24). When synthesized with judiciously tailored hydrophilic and hydrophobic elements, Janus dendrimers can function as powerful structure-directing amphiphiles, with greater

versatility than simple lipids, surfactants, or block copolymers.

Screening libraries of compounds with systematically varying molecular structure and supramolecular architecture represents one of

the most powerful contemporary methods for discovering new materials (25). In our experiment, 11 distinct libraries containing a total of 107 uncharged or positively charged amphiphilic Janus dendrimers were synthesized and screened

Fig. 2. (A) Cryo-TEM of dendrimerosomes from (3,4)12G1-PE-(3,5)-3EO-G1-(OCH₃)₄ in ultrapure water formed by ethanol injection and (B) their 3D intensity profile. (C) Fluorescence microscopy image of a giant dendrimerosome assembled from (3,5)12G1-PE-(3,4)3EO-(OH)₄, encapsulating both hydrophobic Nile red and hydrophilic calcein dyes. (D) Microscopy image and (F) 3D intensity profile of giant dendrimerosome from (3,4)12G1-PE-BMPA-G2-(OH)₈ visualized with Nile red. (E) Giant dendrimerosome from (3,4,5)12G1-PE-(3,4,5)3EO-G1-(OH)₆ visualized with Nile red and calcein. (G) Micropipette aspiration assessment of mechanical strength by micro deformation under negative pressure of a (3,5)12G1-PE-BMPA-G2(OH)₈ giant dendrimerosome. (H) The same dendrimerosome under negative pressure showing small deformation of the membrane. White arrow indicates the deformation of the membrane under micropipette suction pressure. (I) Areal strain determined from micropipette aspiration upon rupture of the same dendrimerosome (α_c). The asterisk indicates the point of membrane failure at the critical areal strain.

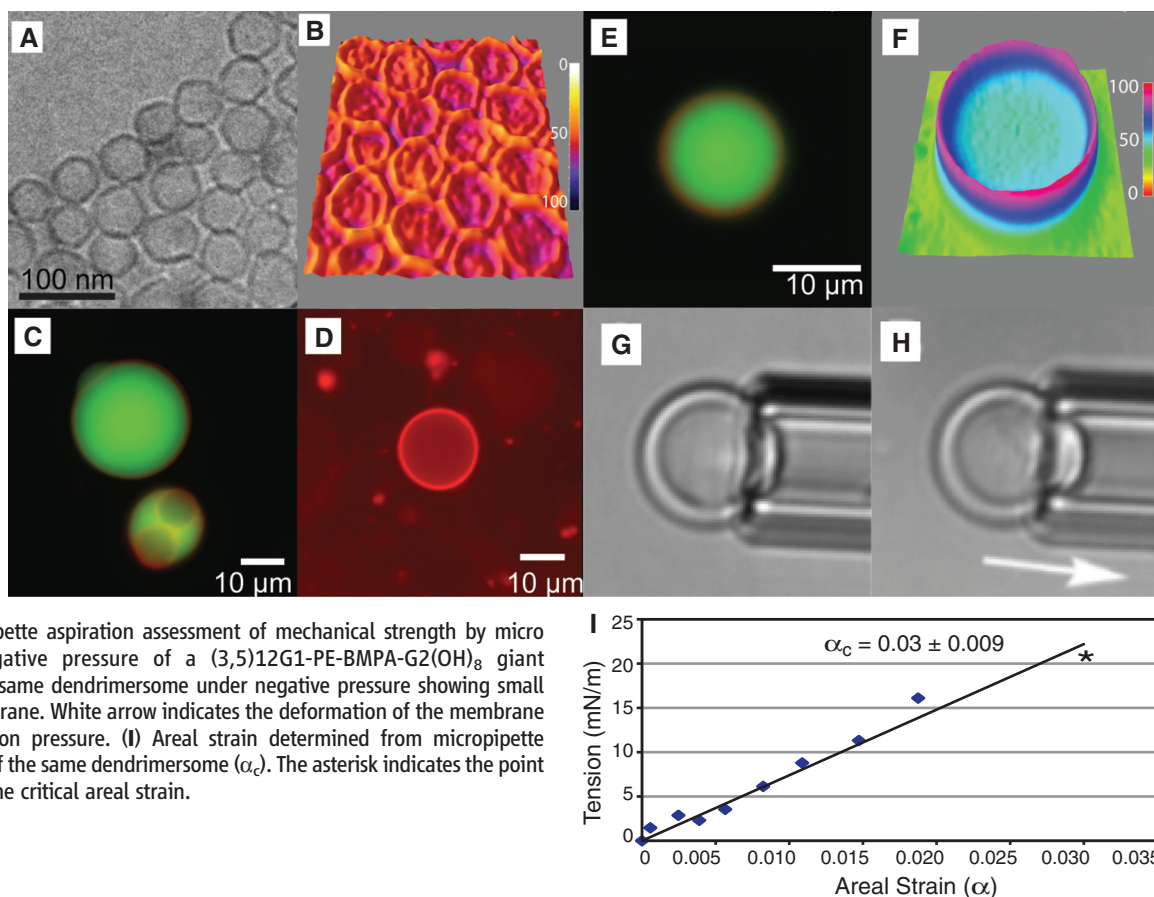
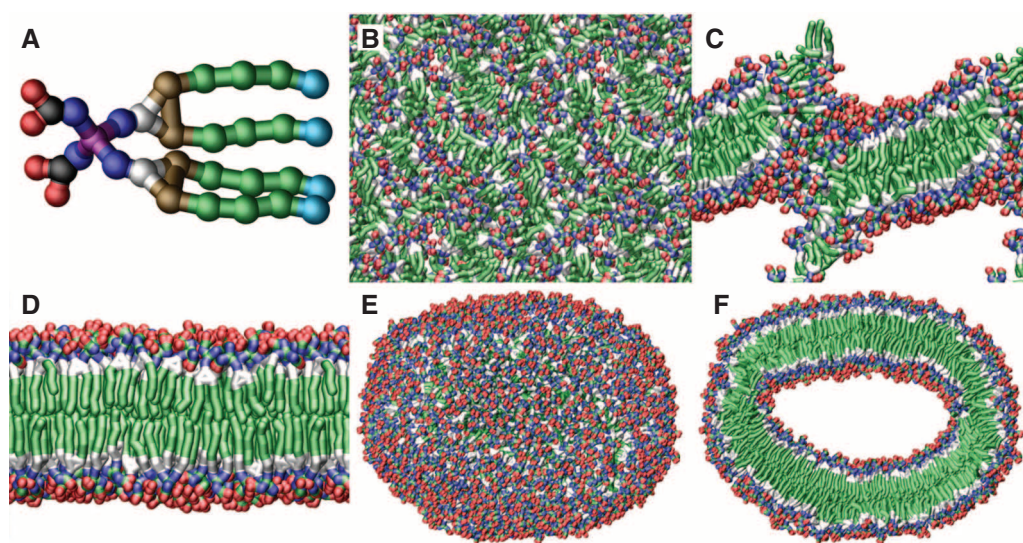


Fig. 3. (A) Molecular model used for the self-assembly Janus dendrimer (3,5)12G1-PE-BMPA-(OH)₄. (B) Self-assembly of (3,5)12G1-PE-BMPA-(OH)₄ using CG molecular dynamics. The CG interaction potentials were derived from fitting to an all-atoms simulation of dendrimer bilayer. Spontaneous bilayer structure formation occurs on a multi-nanosecond time scale using a relatively small number of amphiphiles in the simulation box. The initial snapshot, showing isotropic mixing of dendrons at $t = 0$ ns, is shown here. (C) Snapshot of the simulation at $t = 20$ ns showing lamellar structure formation. (D) Complete simulation showing spontaneous formation of bilayer at $t = 40$ ns. (E) Complete simulation showing spontaneous vesicle formation at $t = 80$ ns. (F) Cut-away view of (E), showing the hollow core of the vesicle. The solvent has been removed for visual clarity. Red, hydroxyl; black, hydrophobic fragment of 2,2-bis(hydroxymethyl)propionate; dark purple, ester; magenta, neopentyl fragment; gray, benzene ring fragment; brown, dioxybenzene ring fragment; green, alkane fragment; blue, terminal alkyl.



for supramolecular assembly in water. These Janus dendrimers (Fig. 1) were designed from AB_3 and constitutionally isomeric AB_2 building blocks, incorporating both hydrophilic and hydrophobic segments that can be rapidly combined to produce diverse sets of exact and monodisperse structures (26). Janus dendrimers were prepared with a combination of convergent approaches for the hydrophobic parts, and divergent or convergent methods for the hydrophilic parts (schemes S1 to S16) (26). Two hydrophobic segments (one aliphatic and one mixed aliphatic-aromatic) and six hydrophilic segments derived from oligoethylene oxide, dimethylolpropionic acid, glycerol, thioglycerol, *tert*-butylcarbamate, and quaternary ammonium salts were combined to yield the portfolio of compounds illustrated in Fig. 1. This simple modular concept allows the fractions of the hydrophilic and hydrophobic portions of the molecule to be varied systematically. Such synthetic versatility and control over molecular architecture and functionality make Janus dendrimers competitive with traditional surfactants and diblock copolymers (9–17).

All of the Janus dendrimers shown in Fig. 1 can be dispersed by injection (17) from dilute solution (typically 10 to 20 mg/mL in ethanol, but also tetrahydrofuran, acetone, or dimethyl sulfoxide) into water. The resulting particle dispersions were characterized for basic morphology by cryogenic transmission electron microscopy (cryo-TEM) and sorted into two categories: (i) dendrimerosome (vesicle)-forming assemblies and (ii) more complex supramolecular assemblies. The wide spectrum of supramolecular architectures that we observed illustrates the unique self-assembly characteristics of Janus dendrimers.

Figure 2, A and B, illustrates a cryo-TEM image of small spherical dendrimerosomes derived from (3,4)12G1-PE-(3,5)-3EO-G1-(OCH₃)₄ (library 2 in Fig. 1). Similar images were obtained from numerous other Janus dendrimers, each exhibiting a unilamellar domain boundary with bilayer thicknesses ranging from 5 to 8 nm (table S4). A notable feature of this picture is the narrow range of dendrimerosome sizes. To more quantitatively establish the size, polydispersity, and stability of the dendrimerosomes, we characterized aqueous dispersions by dynamic light scattering as a function of concentration, temperature, and time. A typical ethanol solution (10 mg/mL) injection into water (2 mL, final concentration = 0.5 mg/mL) produced dendrimerosomes with mean radii of 33 to 732 nm (figs. S15 to S21) and polydispersities (normalized second cumulants) mostly ranging from 0.02 to 0.20 (figs. S15 to S21) with an upper limit of 0.53 (here, 0 corresponds to a perfectly uniform size distribution). Some of these assemblies were stable for long periods of time at room temperature or even when annealed from 22° to 80°C (figs. S2 and S3). Dendrimerosomes derived from library 1 showed a dependence of both size and polydispersity on the concentration in ethanol before injection (table

S2), whereas those associated with libraries containing oligoethyleneoxide in the hydrophilic part of the molecule (for example, library 2) were quite insensitive to these parameters.

Giant dendrimerosomes, 2 to 50 μm in diameter, were prepared by hydration experiments with ultrapure water or phosphate-buffered saline. Giant dendrimerosomes provide access to measurements complimentary (27) to those performed by cryo-TEM on small dendrimerosomes. These experiments are required to establish the mechanical integrity and permeability of the dendrimerosomes, as well as for comparison of these critical

features with liposomes (26) and polymersomes (10–13, 26). In a typical formulation, a 200- μL aliquot of Janus-dendrimer solution in methylene chloride (10 mg/mL) was uniformly deposited on the surface of a roughened Teflon (DuPont, Wilmington, DE) plate, placed in a vial, and then the solvent evaporated over the course of 12 hours. Addition of water and hydration at 60°C for 12 hours results in the formation of giant unilamellar dendrimerosomes. Addition of a molecular dye (for instance, calcein) or drug (e.g., doxorubicin) (7) during the hydration step permits encapsulation within the dendrimerosomes during self-assembly.

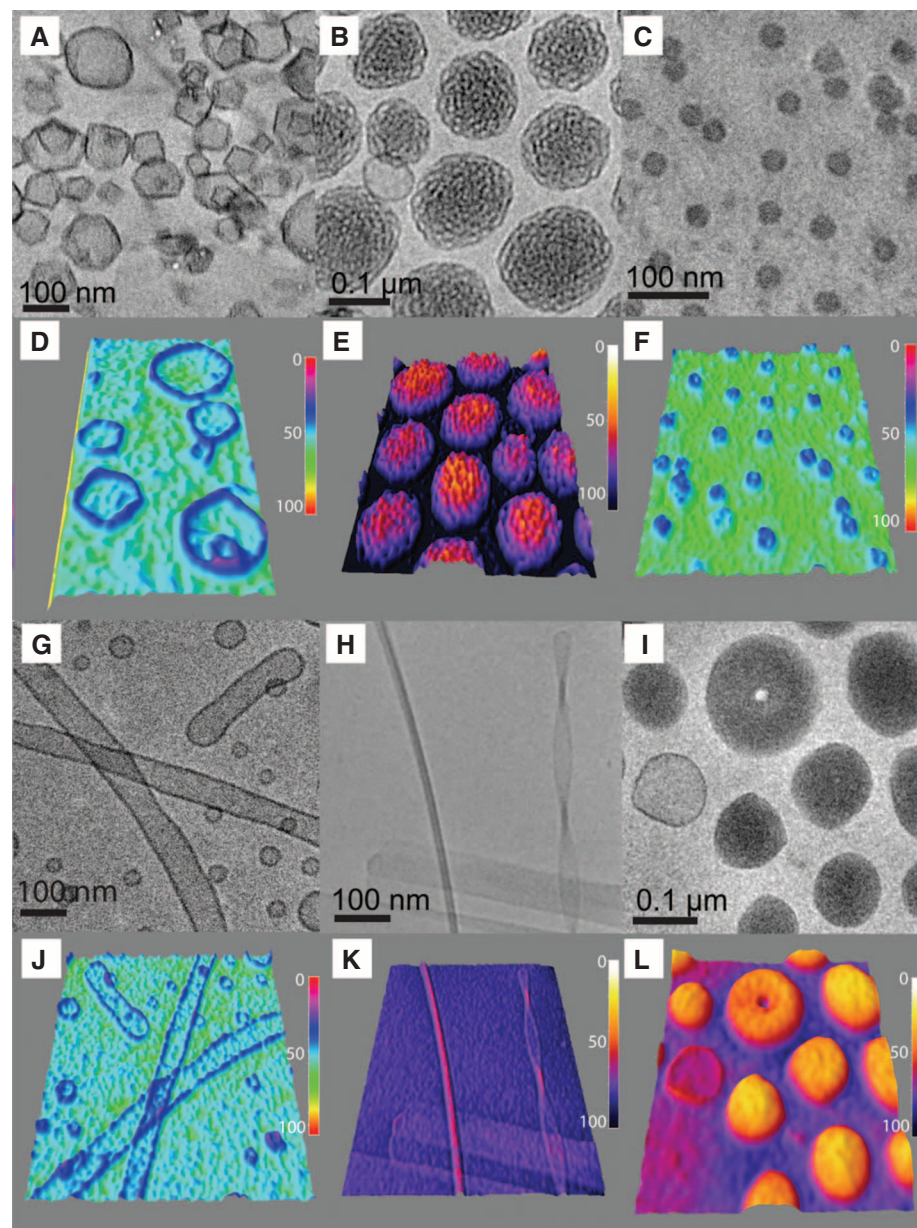


Fig. 4. Cryo-TEM and 3D intensity profiles of (A and D) polygonal dendrimerosomes from (3,4)12G1-PE-(3,4)-3EO-G1-(OMe)₄. (B and E) Bicontinuous cubic particles co-existing with low concentration of spherical dendrimerosomes from (3,5)12G1-PE-(3,4,5)-2EO-(OMe)₆. (C and F) Micelles from (3,4,5)12G1-PE-BMPA-G2-(OH)₈. (G and J) Tubular dendrimerosomes from (3,5)12G1-PE-(3,4,5)-3EO-(OMe)₆. (H and K) Rodlike, ribbon and helical micelles from *tris*12-PE-BMPA-G2-(OH)₈. (I and L) Disklike micelles and toroids from (3,4,5)12G1-PE-(3,5)-3EO-(OMe)₄.

Visualization of both the wall and cavity of giant dendrimersomes was accomplished by fluorescence microscopy after encapsulation of hydrophobic (Nile red) and hydrophilic (calcein) dyes. We observed the hydrophobic dye to localize exclusively in the wall, whereas the hydrophilic dye was noted only in the aqueous interior (Fig. 2, C to F). Micromanipulation experiments with the use of an established micropipette aspiration technique (Fig. 2, G to I) (26) revealed robust mechanical stability: The areal expansion moduli can be tuned over a wide range, $42 \leq K_a \leq 976$ mN/m (table S5), with lipidlike critical areal strains, $0.03 \leq \alpha_c \leq 0.06$ (K_a , areal expansion modulus; α_c , critical areal strain). The strongest dendrimersomes outperform unmodified liposomes by a wide margin ($K_a \leq 234$ mN/m) and are competitive with cholesterol-stabilized liposomes [$K_a = 781$ and 1286 mN/m with 50 and 78% loading of cholesterol in 1-stearoyl-2-oleoyl phosphatidylcholine (SOPC), respectively] (26). Dendrimersomes are considerably stronger, although less compliant, than the toughest polymersomes ($K_a \leq 140$ mN/m and $\alpha_c \geq 0.19$) (10).

The thickness, impermeability, and mechanical properties of giant dendrimersomes indicate that they are excellent candidates for models of biological membranes (1, 3, 6, 13, 16, 18, 19, 27). A membrane thickness of 5 to 8 nm (table S4) should be ideal for incorporating pore-forming proteins, such as melittin (28). This has proven difficult to achieve with polymersomes (13) due to greater membrane thicknesses (generally >8 nm). Melittin incorporation into dendrimersomes was investigated with Janus dendrimers from library 1 and a phospholipid (SOPC) control liposome formed by standard film hydration (figs. S11 and S12). Dendrimersomes containing the fluoro-

cent dye 1-aminonaphthalene-3,6,8-trisulfonate (ANTS) and the quencher α, α' -dipyridinium *p*-xylene dibromide, which quenches ANTS fluorescence at high concentrations (26), were prepared by film-hydration experiments. After the addition of melittin, a dramatic increase in ANTS fluorescence was observed, which is associated with the release of the dye due to melittin-induced pore formation. Subsequent lysis of the vesicles with Triton-X revealed that 60% of the dye was released after melittin incorporation (fig. S11). This experiment demonstrates that, although dendrimersomes have the stability and mechanical properties found with polymersomes, they simultaneously exhibit the structure and function of stabilized phospholipid liposomes.

Dendrimersome stability with time was investigated in biologically relevant media by the formation of membranes via ethanol injection into both citrate buffer and phosphate-buffered saline. Those from library 1 are stable in citrate buffer over a period of 2 weeks and in ultrapure water up to at least 244 days at room temperature (fig. S3). Dendrimersomes from library 2 exhibit excellent stability in both ultrapure water and citrate and phosphate buffers. Selected dendrimersomes from libraries 1 and 2 were loaded with the anticancer drug doxorubicin (7, 8) by hydration of Janus-dendrimer films. Negligible release was observed at physiological temperature and pH (~7.2 to 7.4), again demonstrating the impermeability of dendrimersomes (fig. S13). Predictably, under acidic conditions (pH ~ 5.2 to 5.4), the aromatic-aliphatic ester groups of the dendrimersome cleave, resulting in release of the drug (fig. S13), thereby suggesting pathways to engineer these encapsulants for targeted intracellular drug delivery (8, 9).

Collectively, these results provide powerful evidence that Janus dendrimers produce bilayer vesicles with morphologies and properties competitive with the most advanced liposomes and polymersomes. Architecturally, this category of amphiphiles is qualitatively different than classical lipids or block copolymers. Self-assembly of amphiphilic compounds is a complex subject that remains the target of extensive experimentation and theory. Conceptually, diblock copolymers are most easily understood because the formation of bilayer vesicles, cylindrical micelles, and spherical micelles is rooted in statistically averaged properties (29). A specific morphology reflects the thermodynamically most stable interfacial curvature, which is governed by a competition between interfacial tension and the volume occupied by the hydrophobic and hydrophilic blocks. Interfacial packing can be crudely modeled with the use of simple geometric objects, such as wedges or cones, that account for molecular space filling. This geometric approach also is widely applied to lipids and surfactants (30), which form predominately bilayer vesicles, spherical micelles, and occasionally cylindrical micelles. Tinkering with the shape and size of a lipid or block copolymer influences the spontaneous curvature and, hence, the morphology. Unlike the uniformly sized dendrimersomes reported here, lipids and surfactants rarely produce low polydispersity vesicles, and diblock copolymers never do by simple injection (14, 15). Jung *et al.* (31) have reported that a large bending modulus and a spontaneous curvature are both required to obtain a population of uniform vesicles at equilibrium. This has been achieved only with certain surfactant mixtures.

Controlled branching introduces a powerful new factor in the design of self-assembling amphiphiles. Hydrophobic and hydrophilic chemical functionality can be precisely positioned within Janus dendrimers, resulting in unprecedented control over molecular shape and surface properties. These compounds contain a rich three-dimensional (3D) molecular structure that is manifested in the shape, size, strength, and chemical functionality of the resulting assemblies. Selected Janus dendrimers tagged with Texas red dye on the periphery were shown to coassemble into fluorescent giant unilamellar dendrimersomes with unlabeled Janus dendrimers, block copolymers, and phospholipids (scheme S17 and figs. S7 to S10). This indicates the potential utility of tagged Janus dendrimers in therapeutics for detection and treatment of diseases.

Application of conventional geometric models to the Janus dendrimers is unwarranted given the molecular complexity of these compounds. To gain insight into the self-assembly process, we have therefore employed computer simulations based on coarse-grained (CG) molecular dynamics (32). For example, this approach was applied to Janus dendrimers (3,5)12G1-PE-BMPA-G1-(OH)₄ and (3,5)12G1-PE-BMPA-G2-(OH)₈ known to form dendrimersomes on the basis of cryo-TEM.

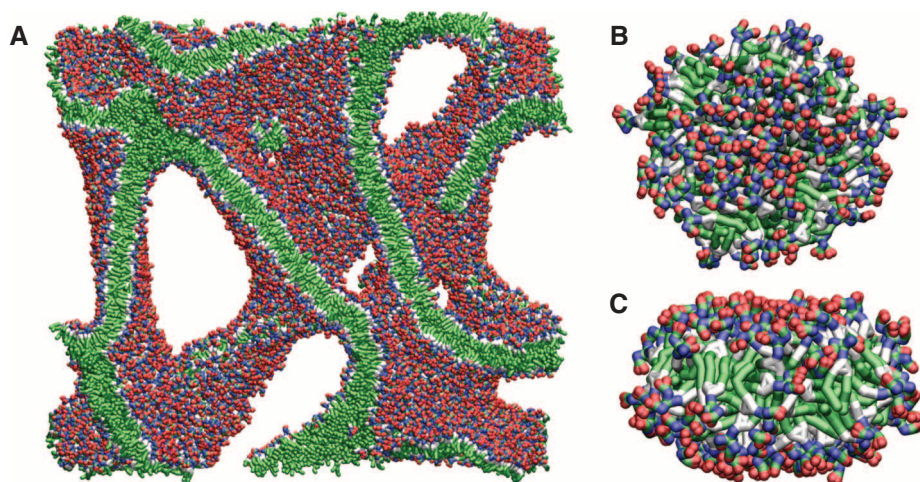


Fig. 5. Self-assembly of the Janus dendrimer (3,5)12G1-PE-BMPA-(OH)₄ using CG molecular dynamics and a large number of dendrimers. (A) Spontaneous and persistent bicontinuous phase occurs within 200 ns when modeled with a ratio of ~133 water molecules per Janus dendrimer. (B and C) Top view and side view, respectively, of a disklike micelle that assembles within 400 ns when the same dendrimer is modeled in a more dilute system having ~808 water molecules per dendrimer. The solvent has been removed for visual clarity.

CG interaction potentials were derived from fits to all-atom simulations of the Janus-dendrimer bilayers. Figure 3 displays snapshots of the resulting self-assembly process, beginning with the disordered state and culminating with the formation of a dendrimerosome on a nanosecond time scale. These computer simulations therefore reinforce the interpretation of the experiments.

Additional evidence supporting the contention that Janus dendrimers are qualitatively different than conventional surfactants and diblock copolymers may be found in other structures illustrated in cryo-TEM images derived from several compounds listed in Fig. 1. Figure 4 illustrates a collection of nanoscale morphologies selected from many that we have documented. Figure 4 (along with Fig. 2A) illustrates sensitivity to systematic structural variations within library 2. Close inspection of Fig. 4A [(3,4)12G1-PE-(3,4)-3EO-G1-(OMe)₄] reveals faceted, polygonal (33) dendrimerosomes, which probably reflects an intriguing compromise between amphiphile-driven bilayer formation and molecular rigidity associated with the branched core. Figure 4B [(3,4)12G1-PE-(3,4)-2EO-G1-(Me)₄] shows particles known as cubosomes (34), which contain a bicontinuous internal morphology. Disklike (35) and toroidal dispersions are found within Fig. 4I [(3,4,5)12G1-PE-(3,5)-3EO-(OMe)₄], whereas (3,5)12G1-PE-(3,4,5)-3EO-(OMe)₆ generates tubular dendrimerosomes (Fig. 4G) (36). Two final examples, drawn from library 2 [(3,4,5)12G1-PE-(3, 5)-3EO-(OMe)₆] and library 8 [*tris*12-PE-BMPA-G2-(OH)₈] are presented in Fig. 4, C and H, evidencing spherical micelles and flat, helical (twisted) ribbons, respectively. Course-grained molecular dynamics computer simulation is capable of capturing elements of this complexity, as illustrated in Fig. 5. These images demonstrate spontaneous self-assembly of (3,5)12G1-PE-BMPA-(OH)₄ into bicontinuous and disklike ob-

jects, depending on the level of hydration, with marked resemblance to morphologies found in Fig. 4, B, E, I, and L.

Though it would be premature to draw detailed conclusions regarding the relationship between specific molecular variations and resulting self-assembled structure at this time, it is clear that Janus dendrimers provide a rich palette for the engineering of new and exciting materials by self-assembly.

References and Notes

- S. J. Singer, G. L. Nicolson, *Science* **175**, 720 (1972).
- A. D. Bangham, M. M. Standish, J. C. Watkins, *J. Mol. Biol.* **13**, 238 (1965).
- M. M. Hanczyc, J. W. Szostak, *Curr. Opin. Chem. Biol.* **8**, 660 (2004).
- M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature* **369**, 301 (1994).
- V. Percec *et al.*, *Nature* **430**, 764 (2004).
- C. K. Haluska *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 15841 (2006).
- O. C. Farokhzad, R. Langer, *ACS Nano* **3**, 16 (2009).
- Y. Barenholz, *Curr. Opin. Colloid Interface Sci.* **6**, 66 (2001).
- X. Guo, F. C. Szoka Jr., *Acc. Chem. Res.* **36**, 335 (2003).
- B. M. Discher *et al.*, *Science* **284**, 1143 (1999).
- D. E. Discher, A. Eisenberg, *Science* **297**, 967 (2002).
- S. F. M. van Dongen *et al.*, *Chem. Rev.* **109**, 6212 (2009).
- K. Kita-Tokarczyk, W. Meier, *Chimia (Aarau)* **62**, 820 (2008).
- A. Rank, S. Hauschild, S. Förster, R. Schubert, *Langmuir* **25**, 1337 (2009).
- J. Thiele, D. Steinhäuser, T. Pfohl, S. Förster, *Langmuir* **26**, 6860 (2010).
- T. F. Zhu, J. W. Szostak, J. Liu, *PLoS ONE* **4**, e5009 (2009).
- R. H. Bridson *et al.*, *J. Pharm. Pharmacol.* **58**, 775 (2006).
- H. Ringsdorf, B. Schlarb, J. Venzmer, *Angew. Chem. Int. Ed. Engl.* **27**, 113 (1988).
- J. L. Thomas, D. A. Tirrell, *Acc. Chem. Res.* **25**, 336 (1992).
- D. D. Lasic, D. Papahadjopoulos, *Science* **267**, 1275 (1995).
- M. P. Krafft, *Adv. Drug Deliv. Rev.* **47**, 209 (2001).
- B. M. Rosen *et al.*, *Chem. Rev.* **109**, 6275 (2009).
- C. C. Lee, J. A. MacKay, J. M. J. Fréchet, F. C. Szoka, *Nat. Biotechnol.* **23**, 1517 (2005).
- R. Esfand, D. A. Tomalia, *Drug Discov. Today* **6**, 427 (2001).
- B. M. Rosen *et al.*, *J. Am. Chem. Soc.* **131**, 17500 (2009).
- Materials and methods are available as supporting material on Science Online.
- H.-G. Döbereiner, *Curr. Opin. Colloid Interface Sci.* **5**, 256 (2000).
- L. Yang, T. A. Harroun, T. M. Weiss, L. Ding, H. W. Huang, *Biophys. J.* **81**, 1475 (2001).
- M. W. Matsen, F. S. Bates, *Macromolecules* **29**, 7641 (1996).
- J. Israelachvili, *Intermolecular and Surface Forces* (Academic Press, London, ed. 2, 1992).
- H. T. Jung, B. Coldren, J. A. Zasadzinski, D. J. Iampietro, E. W. Kaler, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1353 (2001).
- M. L. Klein, W. Shinoda, *Science* **321**, 798 (2008).
- M. Dubois *et al.*, *Nature* **411**, 672 (2001).
- M. Almgren, K. Edwards, G. Karlsson, *Colloid Surf. A: Phys. Eng. Asp.* **174**, 3 (2000).
- A. Walter, P. K. Vinson, A. Kaplun, Y. Talmon, *Biophys. J.* **60**, 1315 (1991).
- S. Chiruvolu *et al.*, *Science* **266**, 1222 (1994).
- We thank the NSF (grant DMR-0548559), NSF-funded Materials Research Science and Engineering Centers at the University of Pennsylvania and at the University of Minnesota, the Academy of Finland, and the P. Roy Vagelos Chair at the University of Pennsylvania for financial support. We also thank T. Tuttila, N. Kuuloja, and M. Lahtinen from the University of Jyväskylä for contributions on the synthesis and analysis of some Janus dendrimers from library 1. Additional funding was provided by the Finnish Cultural Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/1009/DC1
Materials and Methods
SOM Text
Figs. S1 to S21
Schemes S1 to S17
Tables S1 to S8
References

4 December 2009; accepted 14 April 2010
10.1126/science.1185547

Lopsided Growth of Earth's Inner Core

Marc Monnereau,^{1*} Marie Calvet,¹ Ludovic Margerin,² Annie Souriau¹

Hemispherical asymmetry is a prominent feature of Earth's inner core, but how this asymmetry relates to core growth is unknown. Based on multiple-scattering modeling of seismic velocity and attenuation measurements sampling the whole uppermost inner core, we propose that the growth of the solid core implies an eastward drift of the material, driven by crystallization in the Western Hemisphere and melting in the Eastern Hemisphere. This self-sustained translational motion generates an asymmetric distribution of sizes of iron crystals, which grow during their translation. The invoked dynamical process is still active today, which supports the idea of a young inner core.

The inner core of Earth is one of the most enigmatic parts of our planet. This solid body at the center of Earth, with a radius of 1220 km, grows from the crystallization of the iron in the liquid outer core at a present rate of about 0.5 mm/year, a process that has been

occurring for around 1 billion years (1). The structure of the inner core is mostly known from the propagation of seismic waves and from the normal modes that are excited after large earthquakes (2, 3). The inner core is anisotropic, with faster propagation and stronger attenuation for

seismic paths parallel to Earth's rotation axis than for paths parallel to the equatorial plane. However, the uppermost 100 km are nearly isotropic and strongly attenuating, with lower seismic *P*-wave velocity and attenuation in the Western Hemisphere than in the Eastern Hemisphere.

Previous studies report hemispherical anomalies for seismic *P* waves propagating through the uppermost isotropic inner core (called PKIKP waves), but these anomalies exhibit notable differences in their amplitude and spatial distribution (4–7). To resolve some of these in-

¹Université de Toulouse-CNRS, Observatoire Midi-Pyrénées, 14 Avenue Edouard Belin, 31400 Toulouse, France. ²Centre Européen de Recherche et d'Enseignement des Géosciences de l'Environnement, Université Aix-Marseille III-CNRS, 13545 Aix-en-Provence Cedex 04, France.

*To whom correspondence should be addressed. E-mail: marc.monnereau@atp.obs-mip.fr

Fig. 1. (A) Paths of the PKiKP wave (solid line) refracted inside the inner core and the PKiKP wave (dashed line) reflected at the inner-core boundary at distance 140° . (B) Examples of records corresponding approximately to the same epicentral distance (140°) but to increasing distances Δ_G of their turning point to point G [see (C)]. Solid circles are PKiKP waves; open circles are PKiKP waves. Travel-time differences increase with increasing Δ_G values. Data are vertical components, band-pass filtered at 0.7 to 2.0 Hz. Dates and station codes are given on the right. (C) Map of the differential travel-time residuals PKiKP-PKiKP plotted at the ray turning points (Mollweide projection). Solid circles are best-quality data (δ). Contours are plotted every 20° from point G (thick cross) and roughly delineate regions of equal residuals. (D) Differential travel-time residuals and (E) quality factors (in logarithmic scale) as a function of the distance of the ray turning point to point G. Solid circles are best-quality data.

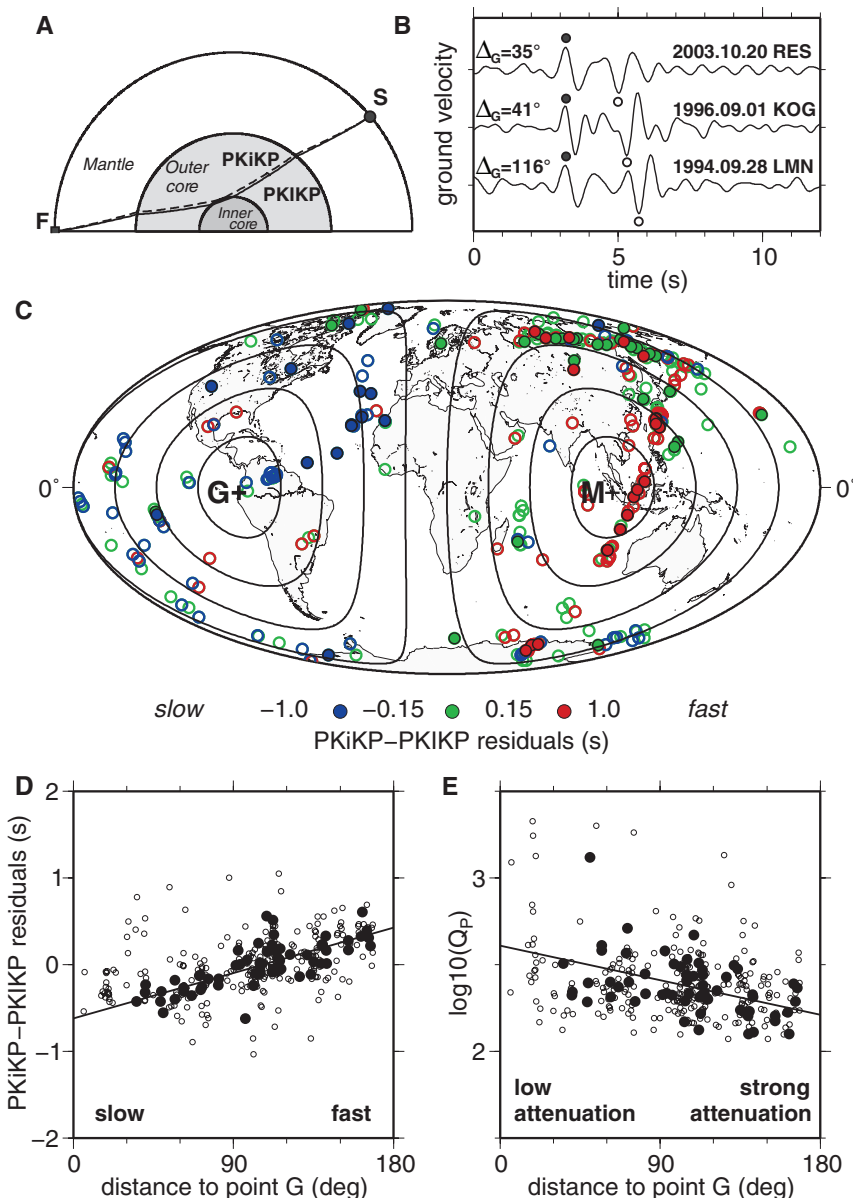


Fig. 2. (A) Relative group-velocity difference between the two hemispheres ($V_M - V_G/V_G$ (color scale) and attenuation ratio Q_M/Q_G (black curves) as a function of the grain sizes a_M and a_G in the uppermost inner core. The elastic constants of the hcp iron model (25) are used to compute attenuation and velocities. We observe that the fast and strongly attenuating Eastern Hemisphere should have larger grains than the slow and less-attenuating Western Hemisphere [$(V_M - V_G)/V_G > 0$ and $Q_M/Q_G < 1$], indicating that crystallization (small grains) occurs in the Western Hemisphere. (B) Most-probable grain size (color scale) at points G and M from a test over 1×10^8 hcp iron crystal models.

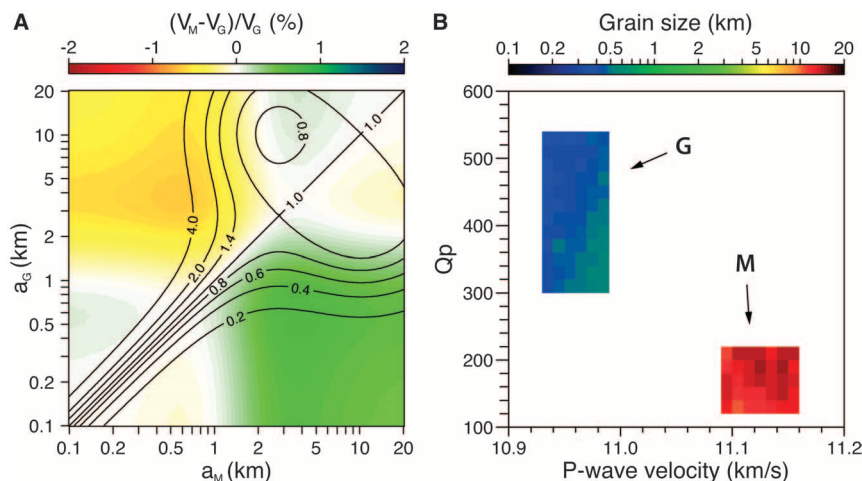
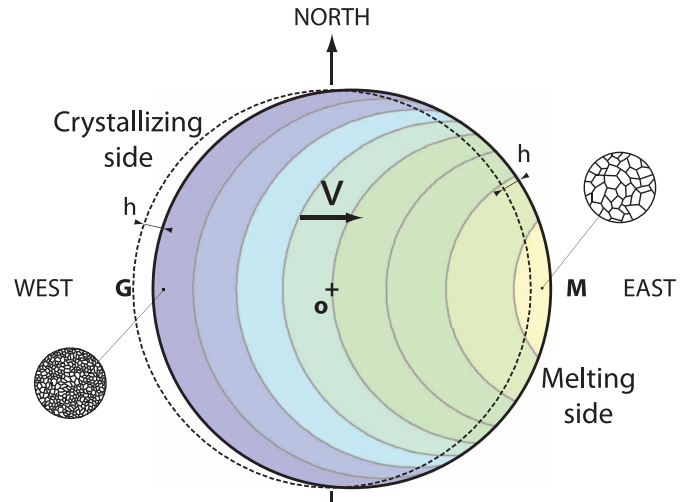


Fig. 3. Schematic cross-section illustrating the inner-core growth model. In a superadiabatic regime, any thermal heterogeneity of harmonic degree-one shifts the center of mass of the inner core toward its colder and denser hemisphere (left). The equilibrium position at the center of mass of Earth (o) is restored by a translation of the inner core (from dashed to solid positions). This induces a topography h that is not in equilibrium with the pressure and temperature conditions within the outer core. Crystallization on the denser hemisphere (left) and melting on the opposite side act to remove the topography, but in return amplify the density heterogeneity and maintain the center of mass shifted toward the crystallizing side. It results in a permanent translation of the inner-core material inside its boundary, whose velocity V is controlled by the phase-change kinetics. The main consequences are an increase of the age (in color) from one side (blue) to the opposite (yellow), and the subsequent grain-size increase, which is compatible with the seismological hemispherical asymmetry.



consistencies, we collected a global data set of PKiKP waves, sampling the uppermost 90 km of the inner core (epicentral distances 135° to 142°), with a particular emphasis on polar regions, which were poorly sampled in previous studies. The PKiKP travel times and amplitudes were measured by comparing them with reference phases reflected at the inner-core boundary (called PKiKP waves) to eliminate mantle and source contributions (8) (Fig. 1, A and B).

The PKiKP-PKiKP travel-time residuals, mapped at the ray turning points (Fig. 1C), reveal an asymmetrical pattern, with high velocities beneath the Eastern Hemisphere and low velocities beneath the Western Hemisphere, as previously observed (4, 5, 7, 9). An expansion of this pattern in spherical harmonics reveals a dominant degree-one with poles close to the equator at longitude $\sim 80^\circ\text{W}$ (point G in Fig. 1C) and its antipode (point M), where GM is a symmetry axis. The travel-time residuals and quality factors (in logarithmic scale) exhibit a linear dependence on angular distance measured from point G (Fig. 1, D and E). These linear relationships confirm that the slower regions are those of lower attenuation and provide numerical constraints for modeling (8).

The observed positive correlation between velocity and attenuation is a distinctive seismic feature of the inner core that is difficult to explain in the framework of viscoelastic attenuation models (10, 11). To explain simultaneously the asymmetry and the correlation between velocity and attenuation, we developed a simple model of iron texture (8). In this model, the inner core is composed of a collection of anisotropic iron grains (cubic or hexagonal) with different sizes and orientations but with an isotropic aspect ratio. The random fluctuations of crystal-axis orientation produce variations in seismic properties from grain to grain, which, in turn, induce attenuation and dispersion of waves by scattering. The quality factor and group velocity of body waves are computed at 1 Hz by

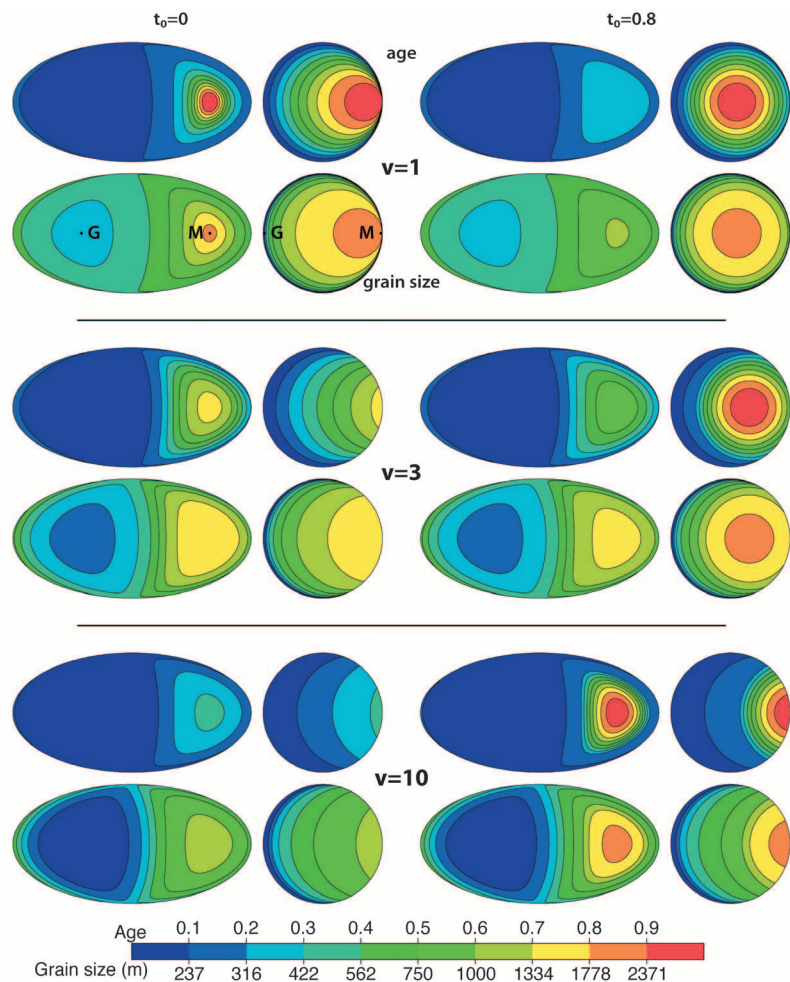


Fig. 4. Age and size of the grains inside the inner core for two initiation times (t_0) of the convection-translation process, normalized by the age of the inner core (left and right panels), and for three translation velocities (v), normalized by the growth rate of the inner core. In the peculiar case $v = 1$, no melting occurs on side M. $t_0 = 0$ corresponds to a translation starting at the beginning of inner-core formation (about 1 billion years ago), $t_0 = 0.8$ to a translation starting at 0.8 times the age of inner-core formation (thus about 200 million years ago). In each subfigure, the top shows the age of the iron grains and the bottom shows their size, represented in map view (Mollweide projection) and cross section. The map views are the average over the uppermost 100 km, as sampled by the seismic paths (Fig. 1).

using a multiple-scattering approach (12). We interpret the dependence of the *P*-wave velocity and attenuation with the distance to the point G as lateral variations of the grain size. This texture model predicts larger grains at point M than at point G (Fig. 2A).

To verify that this result does not depend on the choice of a specific iron model, we performed a systematic exploration in the space of elastic constants for both cubic and hexagonal symmetry. We tested about 1×10^6 models of cubic iron and 1×10^8 models of hexagonal close-packed (hcp) iron; for each model, we allowed the grain size to range from 50 m to 20 km (8). The probability distribution of grain sizes at points G and M for all satisfactory models of hcp iron (Fig. 2B) yields typical grain sizes on the order of 300 to 700 m at point G, and 7 to 15 km at point M. Similar estimates are obtained for cubic iron (8). This grain-size variation accounts for the stronger PKiKP coda observed in the Western Hemisphere (13, 14), because smaller grains backscatter more energy (8).

To interpret such a large contrast of grain size, we propose a growth model that puts forward the role of convection within the inner core. Previous investigations of inner-core convection (15–17) have considered the inner-core boundary as an impermeable surface, such as the core-mantle boundary. However a solid-liquid phase change is clearly a permeable frontier. With such a boundary condition, a uniform velocity field within the inner core is a straightforward solution of the Navier-Stokes equation (8), with the return flow taking place in the outer core. This translational movement necessarily involves permanent crystallization on one side and melting on the opposite side and results in lateral variations of the typical size of iron grains as they grow during their transit time (Fig. 3). The observed hemispherical grain-size distribution indicates that the translation velocity is parallel to the equatorial plane with a west-east orientation (that is, from side G to side M, Fig. 3).

Because no deformation is associated with this flow, no viscous force can balance the buoyancy forces. The equilibrium is simply reached by isostasy, and a small topography (*h*, Fig. 3), resulting from a translation of the inner core, compensates the thermal buoyancy. This translation exposes the colder (that is, denser) side (G) in the crystallizing field and the opposite side (M) in the melting field. Each side no longer corresponds to the solidus, and the topography is thermodynamically unstable. The phase change acts to remove the topography, which is continuously restored by isostatic equilibrium. The inner core is thus involved in a continuous drift from a crystallizing side (G) to a melting side (M), the drift velocity (that is, the convective velocity) being controlled by the phase-change kinetics. Because the iron temperature liquidus increases with pressure, the phase change en-

hances the convection (18). The inner-core boundary position remains stable, except for the radius increase due to the net growth. This convective process generates a cylindrically symmetric structure around the GM axis.

The source of convection is the cooling of the core, but super-adiabatic conditions are necessary. This implies that the cooling rate of the outer core exceeds the conductive heat flux within the inner core. Typically, this occurs for an inner core younger than 1 or 2 billion years, depending on the value of the iron thermal conductivity (15, 19). External conditions also affect the direction of the translation. Because of the columnar nature of the outer-core dynamics (20, 21), heat transport is stronger above equatorial regions of the inner core. As a consequence, convection is favored if the translation takes place in a direction parallel to the equatorial plane, because the vigor of the process is controlled by the ability of the outer core to disperse or provide latent heat released by crystallization or consumed for melting.

The main consequence of our model concerns the age distribution inside the inner core, which is obtained from a simple kinematic model combining the translation mode and the increase of the inner-core radius with time, with the translation velocity serving as the key parameter (8). For velocities greater than the growth rate and a mechanism that is stable in the long term, grains are younger than the inner core, denoting a complete renewal of the inner-core material. In contrast, parts of the primitive inner core are preserved close to the melting side for small translation velocity or late initiation of the mechanism (Fig. 4). A classical model of grain growth (8, 22) predicts grain sizes of a few hundred meters on the crystallizing/growing side, reaching a few kilometers on the melting side, which is in good agreement with the seismological estimates. To account for the seismic asymmetry, the grain size contrast must exceed a factor of 7 and can be as large as a factor of 50. This upper boundary is reached only for a restricted domain of parameters with a transit velocity greater than 100 times the crystallization rate and a very late initiation of the process. In contrast, a factor close to 7 spans a wide range of parameters (fig. S8) but nevertheless always requires a velocity greater than three times the growth rate.

Our convection mechanism proposes an alternative to models where the seismic asymmetry results from outer-flow-induced texture in the Western Hemisphere and columnar dendritic or random texture due to fast growth rate in the Eastern Hemisphere (13, 23). Our model is also compatible with the presence of deep anisotropy. This structural complexity might be related to deformation resulting from forcing by the dominant equatorial mode of inner-core growth (19, 24). The convective translation is not a source of strain but does not prevent such an isostatic adjustment, because both mechanisms are solu-

tions to the flow equation with a permeable boundary.

The west-east translation of the inner core may still be active; otherwise, the ongoing growth of the inner core would restore a uniform grain-size distribution at the inner-core boundary. Because translation operates only in superadiabatic conditions, the inner core may be relatively young.

References and Notes

1. S. Labrosse, J. P. Poirier, J. L. Le Mouél, *Earth Planet. Sci. Lett.* **190**, 111 (2001).
2. A. Souriau, in *Treatise on Geophysics*, G. Schubert, Ed. (Elsevier, Oxford, 2007), vol. 1, pp. 655–693.
3. H. Tkalčić, B. L. N. Kennett, *Aust. J. Earth Sci.* **55**, 419 (2008).
4. R. García, *Geophys. J. Int.* **150**, 651 (2002).
5. A. Cao, B. Romanowicz, *Earth Planet. Sci. Lett.* **228**, 243 (2004).
6. A. Stroujkova, V. F. Cormier, *J. Geophys. Res.* **109**, B10307 (2004).
7. W. C. Yu, L. X. Wen, *Earth Planet. Sci. Lett.* **245**, 581 (2006).
8. Materials and methods are available on Science Online.
9. W. C. Yu, L. X. Wen, *J. Geophys. Res.* **112**, B08304 (2007).
10. X. Li, V. F. Cormier, *J. Geophys. Res.* **107**, 2361 (2002).
11. V. F. Cormier, X. Li, *J. Geophys. Res.* **107**, 2362 (2002).
12. M. Calvet, L. Margerin, *Earth Planet. Sci. Lett.* **267**, 200 (2008).
13. V. F. Cormier, *Earth Planet. Sci. Lett.* **258**, 442 (2007).
14. F. Leyton, K. D. Koper, *J. Geophys. Res.* **112**, B05317 (2007).
15. B. A. Buffett, *Geophys. J. Int.* **179**, 711 (2009).
16. R. Jeanloz, H. R. Wenk, *Geophys. Res. Lett.* **15**, 72 (1988).
17. P. Weber, P. Machetel, *Geophys. Res. Lett.* **19**, 2107 (1992).
18. U. R. Christensen, D. A. Yuen, *J. Geophys. Res.* **90**, 291 (1985).
19. R. Deguen, P. Cardin, *Nat. Geosci.* **2**, 419 (2009).
20. F. H. Busse, *J. Fluid Mech.* **44**, 441 (1970).
21. J. Aubert, D. Brito, H. C. Nataf, P. Cardin, J. P. Masson, *Phys. Earth Planet. Inter.* **128**, 51 (2001).
22. L. Venet, T. Duffar, R. Deguen, *C. R. Geosci.* **341**, 513 (2009).
23. J. Aubert, H. Amit, G. Hulot, P. Olson, *Nature* **454**, 758 (2008).
24. S. Yoshida, I. Sumita, M. Kumazawa, *J. Geophys. Res.* **101**, 28085 (1996).
25. L. Vočadlo, *Earth Planet. Sci. Lett.* **254**, 227 (2007).
26. We thank V. F. Cormier and two anonymous referees for helpful reviews; M. Toplis, T. Alboussière, and R. Deguen for stimulating discussions; and the French group Structure, Evolution et Dynamique de l'Intérieur de la Terre (SEDIT) Noyau for supporting interdisciplinary exchanges. We thank the institutions that provided data for this study through the Incorporated Research Institutions for Seismology. Financial support has been provided by Institut National des Sciences de l'Univers (INSU)–CNRS programs.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1186212/DC1
Materials and Methods
Figs. S1 to S8
References

22 December 2009; accepted 1 April 2010
Published online 15 April 2010;
10.1126/science.1186212
Include this information when citing this paper.

Regional Variation of Inner Core Anisotropy from Seismic Normal Mode Observations

Arwen Deuss,^{1*} Jessica C. E. Irving,¹ John H. Woodhouse²

Earth's solid inner core is surrounded by a convecting liquid outer core, creating the geodynamo driving the planet's magnetic field. Seismic studies using compressional body waves suggest hemispherical variation in the anisotropic structure of the inner core, but are poorly constrained because of limited earthquake and receiver distribution. Here, using normal mode splitting function measurements from large earthquakes, based on extended cross-coupling theory, we observe both regional variations and eastern versus western hemispherical anisotropy in the inner core. The similarity of this pattern with Earth's magnetic field suggests freezing-in of crystal alignment during solidification or texturing by Maxwell stress as origins of the anisotropy. These observations limit the amount of inner core super rotation, but would be consistent with oscillation.

As the Earth continues to cool, the inner core grows by solidification of the fluid outer core (1). Solidification results in the release of light elements and latent heat, which drive the geodynamo generating the Earth's magnetic field (2). The details of the magnetic field and geodynamo depend on the existence and structure of the inner core, which can be studied directly by using seismic data. Seismological observations established that the inner core is anisotropic (3, 4) and possibly rotating faster than the Earth's mantle (5).

Recent seismic studies have suggested the existence of hemispherical variations in anisotropy—the Western Hemisphere displaying much stronger anisotropy than the Eastern Hemisphere (6), which may be related to the thermal evolution of the core-mantle boundary region and the structure of the magnetic field (7). However, hemispherical variations in inner core anisotropy have so far been seen only in compressional body waves (6, 8), which are limited to sampling only a few small regions of the core because of uneven station and earthquake coverage. These potential biases limit their use in making comparisons of inner core structure with the Earth's magnetic field and thermal structure. Hemispherical variations may be too simplistic an interpretation of more complicated laterally varying structure, and we do not know if they also exist in shear waves.

Normal modes—whole Earth oscillations at the lower part of the frequency range (<10 mHz)—have the potential to provide both full global coverage and robust identification of hemispherical variation in inner core anisotropy. However, lack of appropriate theory has prevented the resolution of more complicated structures and re-

gional variations by using this approach. The simplest theory to study normal modes, called self-coupling, assumes that a mode may be treated as isolated (9). All previous normal mode studies have applied self-coupling and have sought only inner core anisotropy that is symmetric around the Earth's rotational axis (4, 10–12). To observe hemispherical (i.e., antisymmetric) variations, it is essential to take cross-coupling between pairs of modes into account (13). Cross-coupling becomes important when two or more normal modes are close in the frequency spectrum and start to resonate (14). We recently extended normal mode theory (15), enabling us to include hemispherical variations in inner core anisotropy, and apply it here to make observations in real data (16). Here, we present normal mode splitting functions measured from normal mode spectra from over 90 large earthquakes (moment magnitude > 7.5) from 1976 to 2009 to observe hemispherical anisotropy and to interpret these observations using model predictions (17). Spheroidal normal modes are denoted ${}_nS_l$, where n is the overtone number and l is the angular order. J denotes modes that are confined to the inner core (fig. S1 and S2).

Normal mode ${}_{16}S_5$ strongly cross-couples to inner core confined mode ${}_{17}S_4^J$. The self-coupling splitting function measurement for ${}_{16}S_5$ (Fig. 1A) confirms earlier measurements (12, 18) in which a symmetric zonal pattern (i.e., constant with longitude), with alternating bands across the polar regions and along the equator, is apparent. This characteristic pattern requires cylindrical inner core anisotropy (12) in addition to heterogeneous mantle structure (Fig. 1B) because calculations for mantle-only structure do not explain the anomalous polar regions. Instead, weaker subduction zone anomalies would be visible beneath the Americas and east Asia (Fig. 1C).

The observed cross-coupled splitting function between ${}_{16}S_5$ and inner core confined mode ${}_{17}S_4^J$ shows an antisymmetric flip in sign across

Africa (Fig. 1D). Calculations, with the use of our previously developed theory (15), for a model with inner core anisotropy in the Western Hemisphere only (fig. S3) show that this flip can be explained by strong hemispherical variation in inner core anisotropy. The negative splitting function anomalies around the polar regions in the Western Hemisphere are due to increased anisotropy there, and the positive splitting function anomalies in the Eastern Hemisphere polar regions are due to locally weaker anisotropy (Fig. 1E). The pattern completely disappears when stripping the hemispherical inner core anisotropy from the predictions, leaving a mantle-only structure (Fig. 1F), confirming that this mode pair is very sensitive to inner core structure.

A range of tests (19), including cross-validation to determine error boundaries in our measurements (fig. S5), show that our observations are robust. Conveniently, the strongest cross-coupling for inner core hemispherical structure is found in normal mode pairs of which one of the modes is an oscillation of the inner core only, a so-called inner core confined mode. An inner core confined mode is not sensitive to the outer core, mantle, or crust (fig. S2) and can cross-couple with another mode only in the presence of inner core structure. Previous studies suggested that normal mode observations of inner core anisotropy may be due to outer core structure instead (20) or that the hemispherical variations seen in compressional waves may be due to anomalous structure in the core-mantle boundary region. By making use of pairs that are sensitive only to hemispherical inner core structure, we show that any observed structure must instead come from the inner core, which provides evidence for the existence of inner core anisotropy. It is also noteworthy that the normal modes observed here are mainly sensitive to the shear wave structure of the inner core; this suggests that regional variations in anisotropy can be observed not only in compressional but also in shear wave velocity.

Several more pairs of cross-coupled modes show the characteristic antisymmetric change in signature across Africa in their cross-coupled splitting functions (Fig. 2). Mode pair ${}_8S_5$ - ${}_{10}S_7^J$ (Fig. 2A) is again a combination of an inner core confined mode with an observable mode. Comparison with model predictions (fig. S4A) shows that the alternating pattern with negative splitting function anomalies near the poles in the Western Hemisphere is due to increased anisotropy and that the anomalies with opposite polarity in the Eastern Hemisphere are due to weaker anisotropy; the observed and predicted patterns are similar. Both constituent modes of the pair ${}_{14}S_4$ - ${}_{11}S_7$ (Fig. 2B) are observable at the Earth's surface, and both are sensitive to mantle and core structure. The cross-coupled splitting function for ${}_{14}S_4$ - ${}_{11}S_7$ is a combination of mantle and core structure, showing much stronger antisymmetric splitting than is predicted for current models of inner core anisotropy (fig. S4B). For this mode pair, the predictions confirm that increased

¹Department of Earth Sciences, University of Cambridge, Cambridge CB3 0EZ, UK. ²Department of Earth Sciences, University of Oxford, Oxford OX1 3PR, UK.

*To whom correspondence should be addressed. E-mail: afd28@cam.ac.uk

anisotropy in the Western Hemisphere shows up as positive splitting function anomalies in the polar regions.

Short-period differential PKPbc-PKIKP and PKPab-PKIKP travel times allow for comparison of splitting functions with body wave observations, revealing inner core structure by using pairs of waves that have similar paths in the mantle and outer core but differ in the inner core (fig. S6). PKIKP is the compressional wave propagating from the mantle into the outer and inner core and returning to the Earth's surface. PKPbc and PKPab are branches of compressional waves that travel only the mantle and outer core. In agreement with previous studies (6), polar paths

show large, positive travel-time anomalies in the Western Hemisphere and smaller anomalies in the eastern hemisphere (Fig. 3). These anomalies support the general interpretation that inner core anisotropy is aligned with the north-south axis in the Western Hemisphere and is weaker in the Eastern Hemisphere. The boundaries between the Eastern and Western Hemispheres at 14°E and 151°W, revealed using a model space search, serve as boundaries for the cross-coupled hemispherical inner core anisotropy predictions (Fig. 1E and fig. S4).

The strongest anisotropy in the PKIKP observations is found in the Western Hemisphere under the Americas. These observations are

dominated by earthquakes in the South Sandwich Islands and stations in Alaska; sampling of other regions by polar paths is much sparser. This

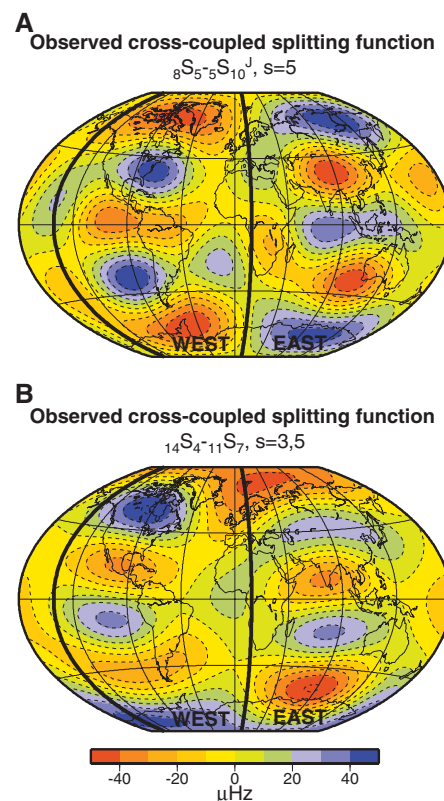


Fig. 2. Observed cross-coupled splitting functions for mode pairs (A) $8S_5-5S_{10}$, and (B) $14S_4-11S_7$.

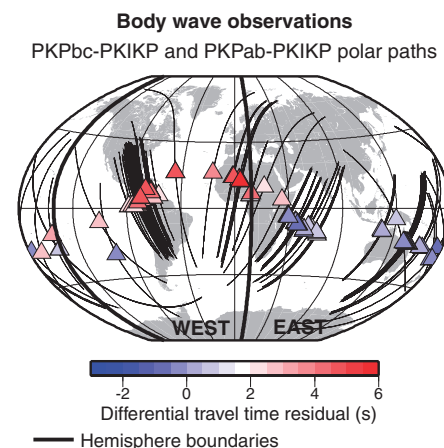


Fig. 3. Body wave observations of PKPbc-PKIKP and PKPab-PKIKP differential travel time residuals for polar paths. Large positive residuals (red triangles) are indicative of anisotropy and are predominately found in the Western Hemisphere, whereas the residuals in the Eastern Hemisphere are much smaller (blue triangles) and show no evidence of inner core anisotropy. Thick lines indicate hemisphere boundaries obtained by a model space search. Thin lines indicate the path of the PKIKP wave through the inner core. Substantial areas of the inner core are poorly sampled, particularly the Northern Hemisphere in Asia and the mid-Pacific region.

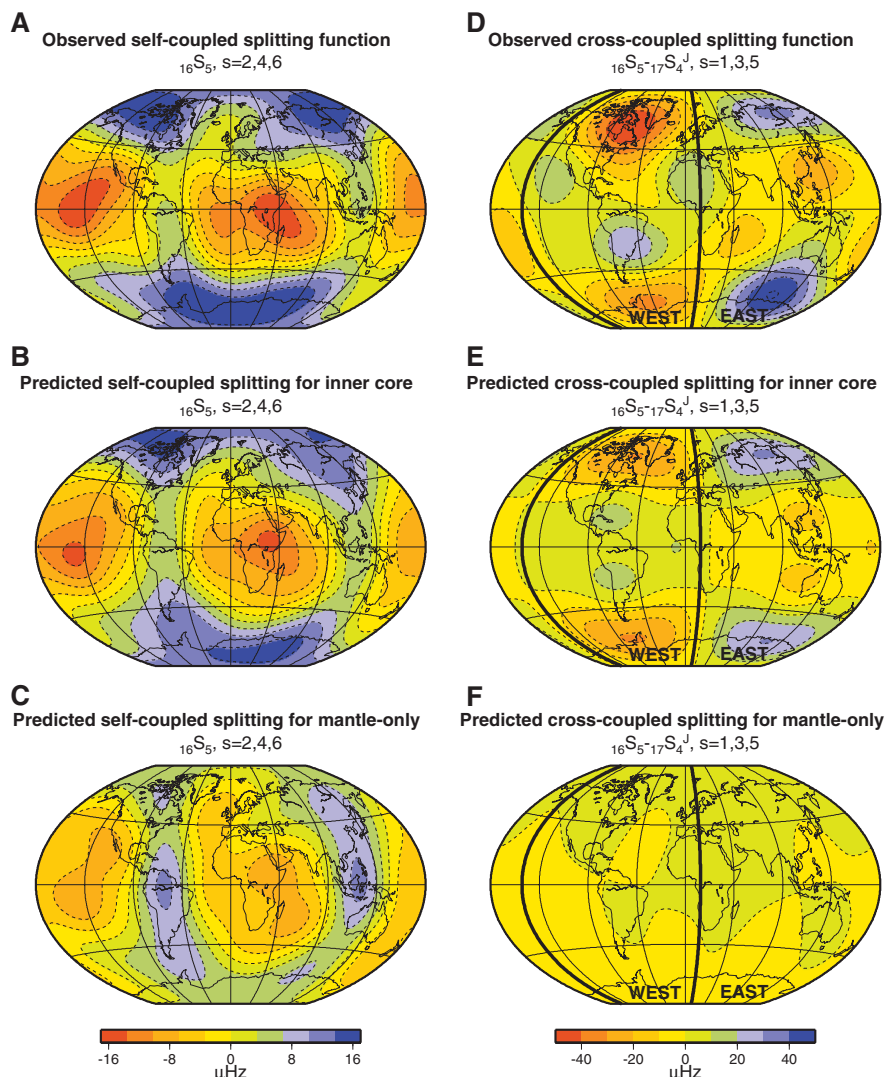


Fig. 1. Observed and predicted splitting functions for the mode pair $16S_5$ and $17S_4$. (A) Observed splitting function using self-coupling only for $16S_5$ showing typical zonal splitting. (B) Predicted self-coupled splitting function for a mantle model and a cylindrically anisotropic inner core model (12). (C) Predicted self-coupled splitting function for mantle-only model S20RTS (31). (D) Observed cross-coupled splitting function showing antisymmetric splitting, which changes sign across Africa. (E) Predicted cross-coupled splitting function for anisotropy only between hemisphere boundaries at 151°W and 14°E. (F) Predicted cross-coupled splitting function for mantle-only structure. Thick lines denote the hemisphere boundaries found from body wave observations (Fig. 3). s is the angular order of the observed structure.

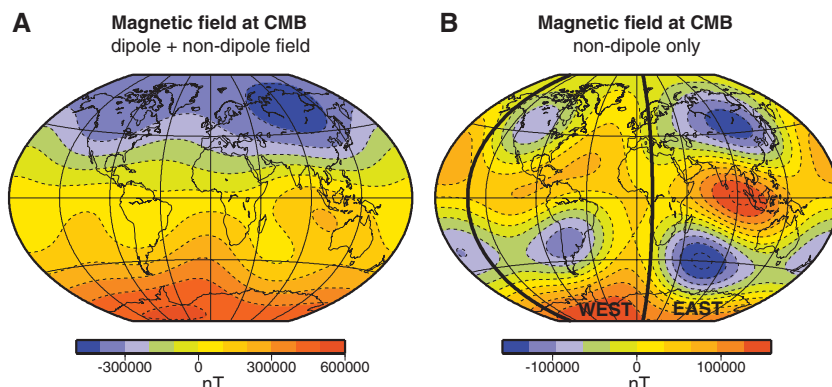


Fig. 4. Observed radial component of the Earth's magnetic field at the CMB, averaged over the last 5 million years (27). **(A)** The total magnetic field is dominated by the dipole component; the nondipole field is also included. **(B)** The nondipole component of the field shows four increased negative flux patches. These flux patches are also seen in the current-day magnetic field (fig. S7) and correspond to the locations of maximum hemispherical variations in seismic anisotropy (Figs. 1D and 2, A and B).

region very closely matches the area of strongest anisotropy found independently in our observations of cross-coupled, normal mode splitting function (Figs. 1D and 2). The normal modes also reveal that the region of weakest anisotropy lies under eastern Asia, an area that is not well covered by PKIKP paths.

Inspecting the observed splitting functions in more detail, we find a pattern of regional variations in addition to the simple Eastern versus Western Hemisphere division in the PKIKP observations. The splitting function predictions for simple hemispheres are antisymmetric across Africa (Fig. 1E and fig. S4), whereas the observations show wide transition zone regions between the narrow regions of strongest and weakest anisotropy. The observations also show regions of variable strength on either side of the hemisphere boundary across Africa (21). For example, ${}_8S_5$ - ${}_5S_{10}^J$ (Fig. 2A) reveals an additional negative frequency region under southern Africa and in the mid-Pacific. Similar additional features are seen in ${}_{16}S_5$ - ${}_{17}S_4^J$ (Fig. 1D) beneath Madagascar.

Inner core anisotropy is acquired either during solidification by freezing-in of crystal alignment (7, 22), or by deformation texturing after solidification due to thermal convection (23), anisotropic growth (24), or the Maxwell stress of the magnetic field (25, 26). The observations reported here allow us to compare models of the average magnetic field (27–29) (Fig. 4) with the seismically observed hemispherical variations and to test these hypotheses. The radial component of the magnetic field at the core-mantle boundary is dominated by the dipole component (Fig. 4A). The nondipole component shows four concentrated flux patches, two in the Eastern Hemisphere and two in the Western Hemisphere (Fig. 4B). The locations of the magnetic flux patches coincide with the regions of strongest anisotropy across the Americas and weakest anisotropy across eastern Asia (comparing Fig.

1D with Fig. 4B). The two flux patches in the Eastern Hemisphere are stronger and are associated with the weakest anisotropy in the same hemisphere. Weak magnetic field regions are seen across Africa and in the Pacific, in agreement with the transition regions between strong and weak anisotropy in normal mode observations.

The similarity between the locations of regional flux concentrations in the radial magnetic field and the strength of the seismic anisotropy suggests that they share a common origin and rules out deformation by thermal convection (23) or anisotropic growth (24). Magnetic flux patches are caused by variations in temperature at the core-mantle boundary, locally extracting more or less heat from the core and thus concentrating upwellings and downwellings in outer core convection (30). Complex convection patterns may then imprint variable alignment during freezing at the inner core boundary (7). The question remains whether the flux patches have been stable for long enough in Earth's history to generate hemispherical differences in the deeper parts of the inner core, but the deeper anisotropy may have been acquired after solidification due to texturing by Maxwell stress (25, 26). Thus, hemispherical variations in seismic anisotropy may help in unraveling the magnetic field of the past.

Inner core super rotation of 0.1° per year (5) would average out hemispherical variations caused by texturing either during or after solidification. We find that the areas of weak and strong anisotropy do not cover a full hemisphere but are narrow and separated by wide transition regions. This might also be explained by inner core oscillation, which would make the boundary between the two hemispheres blurred.

References and Notes

1. B. A. Buffett, H. E. Huppert, J. R. Lister, A. W. Woods, *Nature* **356**, 329 (1992).

2. D. Gubbins, D. Alfe, G. Masters, D. Price, M. Gillan, *Geophys. J. Int.* **155**, 609 (2003).
3. A. Morelli, A. M. Dziewonski, J. H. Woodhouse, *Geophys. Res. Lett.* **13**, 1545 (1986).
4. J. H. Woodhouse, D. Giardini, X.-D. Li, *Geophys. Res. Lett.* **13**, 1549 (1986).
5. X.-D. Song, P. G. Richards, *Nature* **382**, 221 (1996).
6. S. Tanaka, H. Hamaguchi, *J. Geophys. Res.* **102** (B2), 2925 (1997).
7. J. Aubert, H. Amit, G. Hulot, P. Olson, *Nature* **454**, 758 (2008).
8. F. Niu, L. Wen, *Nature* **410**, 1081 (2001).
9. D. Giardini, X.-D. Li, J. H. Woodhouse, *J. Geophys. Res.* **93** (B11), 13716 (1988).
10. C. Beghein, J. Trampert, *Science* **299**, 552 (2003).
11. M. Ishii, J. Tromp, A. M. Dziewonski, G. Ekström, *J. Geophys. Res.* **107** (B12), 2379 (2002).
12. J. J. Durek, B. Romanowicz, *Geophys. J. Int.* **139**, 599 (1999).
13. Self-coupling is sensitive only to even-degree structure for symmetry reasons. Hemispheres are an odd-degree structure, which requires cross-coupling.
14. A. Deuss, J. H. Woodhouse, *Geophys. J. Int.* **146**, 833 (2001).
15. J. C. E. Irving, A. Deuss, J. H. Woodhouse, *Geophys. J. Int.* **178**, 962 (2009).
16. J. S. Resovsky, M. H. Ritzwoller, *J. Geophys. Res.* **103**, (B1), 783 (1998).
17. Materials and methods are available as supporting material on Science Online.
18. X. He, J. Tromp, *J. Geophys. Res.* **101** (B9), 20053 (1996).
19. Adding hemispherical structure improved the data misfit (table S1), proving that such structures are required by the data. We also find that the flip in sign is independent of the starting model used in the inversion and appeared without an a priori imposed boundary there.
20. B. Romanowicz, L. Breger, *J. Geophys. Res.* **105** (B9), 21,559 (2000).
21. W. Yu, L. Wen, *J. Geophys. Res.* **112**, B08304 (2007).
22. M. I. Bergman, *Nature* **389**, 60 (1997).
23. R. Jeanloz, H. R. Wenk, *Geophys. Res. Lett.* **15**, 72 (1988).
24. S. I. Yoshida, I. Sumita, M. Kumazawa, *J. Geophys. Res.* **101** (B12), 28,085 (1996).
25. S. Karato, *Nature* **402**, 871 (1999).
26. B. A. Buffett, H.-R. Wenk, *Nature* **413**, 60 (2001).
27. P. Kelly, D. Gubbins, *Geophys. J. Int.* **128**, 315 (1997).
28. C. L. Johnson, C. G. Constable, *Geophys. J. Int.* **122**, 489 (1995).
29. A. Jackson, A. R. T. Jonkers, M. R. Walker, *Philos. Trans. R. Soc. Lond. A* **358**, 957 (2000).
30. J. Bloxham, D. Gubbins, *Nature* **325**, 511 (1987).
31. J. Ritsema, H. van Heijst, J. H. Woodhouse, *Science* **286**, 1925 (1999).
32. J. Ritsema and H. van Heijst assisted in building the normal mode data set. We appreciate the suggestions by V. Cormier and two anonymous reviewers. The research was funded by the European Research Council (ERC) under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement number 204995.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1188596/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Tables S1 and S2
References

22 February 2010; accepted 8 April 2010
Published online 15 April 2010;
10.1126/science.1188596
Include this information when citing this paper.

The Onset of Collective Behavior in Social Amoebae

Thomas Gregor,^{1*} Koichi Fujimoto,^{2†} Noritaka Masaki,² Satoshi Sawai^{1,2‡}

In the social amoebae *Dictyostelium discoideum*, periodic synthesis and release of extracellular cyclic adenosine 3',5'-monophosphate (cAMP) guide cell aggregation and commitment to form fruiting bodies. It is unclear whether these oscillations are an intrinsic property of individual cells or if they exist only as a population-level phenomenon. Here, we showed by live-cell imaging of intact cell populations that pulses originate from a discrete location despite constant exchange of cells to and from the region. In a perfusion chamber, both isolated single cells and cell populations switched from quiescence to rhythmic activity depending on the concentration of extracellular cAMP. A quantitative analysis showed that stochastic pulsing of individual cells below the threshold concentration of extracellular cAMP plays a critical role in the onset of collective behavior.

Populations of microorganisms often undergo transitions to coordinated activities depending on nutrient availability and presence of other microbes in the environment. In a process termed “quorum sensing” (1), small signaling molecules are synthesized and secreted into the extracellular space. When the concentration of the molecules reaches a threshold, the cell population undergoes a transition to a state that

collectively can cope with the new environment. Studies on chemical oscillators (2), glycolytic oscillations in yeast (3), and a synthetic gene circuit in bacteria (4) have demonstrated that synchronized oscillations may rely on a similar quorum sensing-type transition. Unlike in cases where synchronized oscillations appear by entrainment of phase and frequencies of autonomously oscillating elements [e.g., fireflies (5), circadian

rhythms (6), somite segmentation (7), Josephson junction arrays (8), chemical reactions (9), etc. (10)], the quorum sensing-type transition is accompanied by a spontaneous change from quiescence to oscillations at the individual level. The transition, referred to as “dynamical quorum sensing,” encodes cell-density information in the frequency of the oscillations (2, 3), because the small-molecule threshold may be reached more quickly at higher cell densities. Although the scheme can provide a robust means for a single cell to change its state qualitatively depending on the cell density, exposing the basis of the oscillatory transition in cell populations has remained a challenge (11, 12) because of difficulties in analyzing the dynamics of individual cells and the

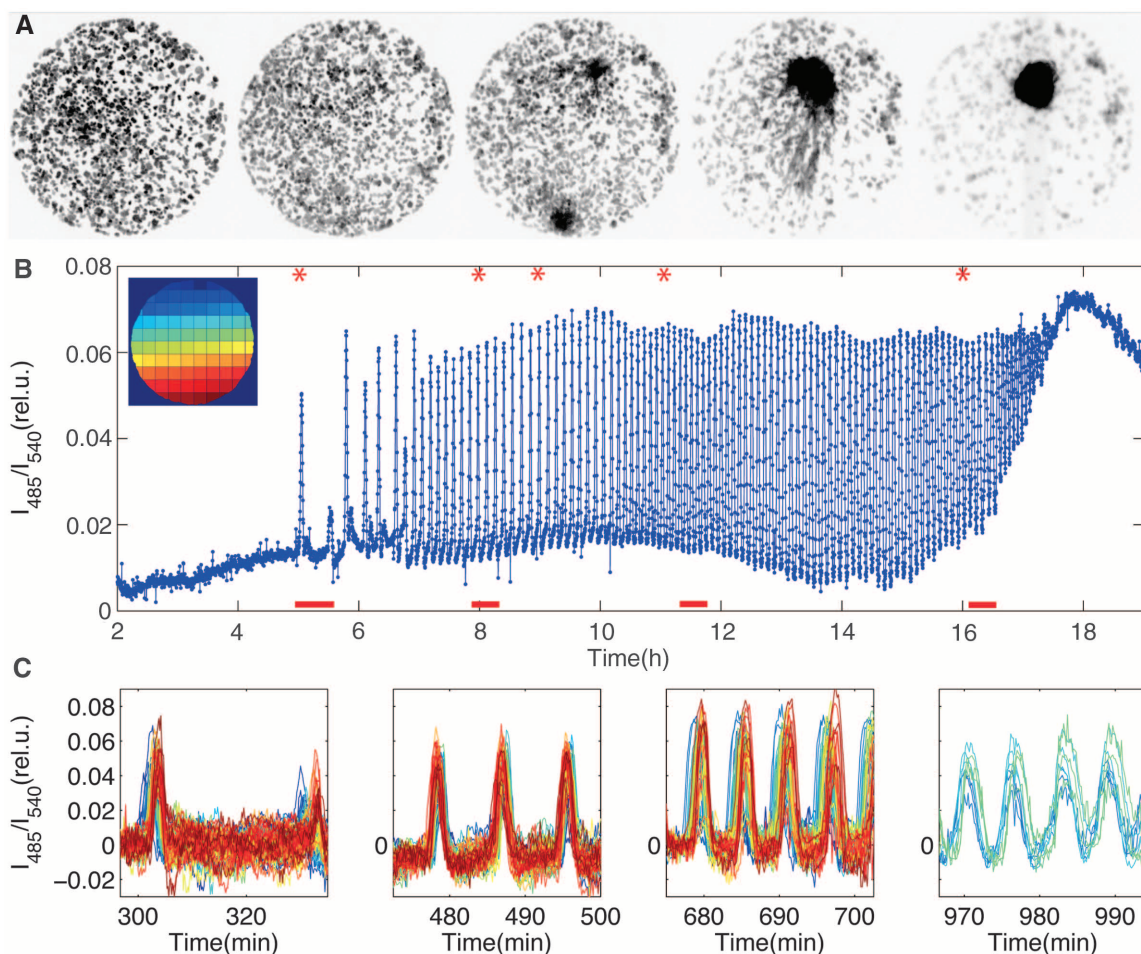
¹Graduate School of Arts and Sciences, University of Tokyo, Tokyo 153-8902, Japan. ²Exploratory Research for Advanced Technology (ERATO) Complex Systems Biology Project, Japan Science and Technology Agency (JST), Tokyo 153-8902, Japan.

*Present address: Department of Physics, Princeton University, Princeton, NJ 08544, USA.

†Present address: Department of Biological Sciences, Osaka University, Toyonaka 560-0043, Japan.

‡To whom correspondence should be addressed. E-mail: cssawai@mail.ecc.u-tokyo.ac.jp

Fig. 1. Live-cell imaging of cAMP signaling during early development of *D. discoideum* cells. (A) About 180 cells were confined to a 420- μ m-diameter area on hydrophobic agar (22). Snapshots were taken at times indicated by the red asterisks in (B). Dark area in rightmost snapshot corresponds to final aggregation site of the population. (B) Time course of changes in FRET efficiency, averaged over all cells (time indicates hours after starvation). The fluorescent intensities of the cyan fluorescent protein channel divided by that of the yellow fluorescent protein channel are plotted on the y axis. The upper left inset is a colored schematic for the subregions analyzed in (C). (C) Enlargements of the time series for each subregion, containing zero to five cells. Red bars indicate time windows in (B), chronologically ordered from left to right. Colors correspond to the colors of the spatial regions displayed in the inset in (B). Small differences in the rising phase of the pulses for individual regions correspond to cAMP waves that are propagating in space (see also fig. S7).



population simultaneously. Moreover, how microorganisms use cellular oscillatory mechanisms to implement complex life-cycle strategies at the population scale remains unexplored.

Chemoattractant signaling in the social amoebae *Dictyostelium discoideum* is one of the best-known examples of cell-cell signaling that mediates cooperation in microorganisms (13).

When stimulated with extracellular cyclic adenosine 3',5'-monophosphate (cAMP), cells respond by synthesizing and secreting more cAMP, which results in nondissipating waves of cAMP (14, 15)

Fig. 2. Synchronized population-level oscillations of cytosolic cAMP. Cells were placed in a perfusion chamber (volume of ~0.25 ml) 4 to 6 hours after starvation. **(A)** Population-averaged behavior at cell densities of 1/8 monolayer (ML; 1 ML unit is 6600 cells/mm²), 1/32 ML, and 1/64 ML (top to bottom, respectively) under buffer at a fixed flow rate of 1 ml/min. The lowest panel is at 1/32 ML and 16 ml/min. **(B)** A phase diagram summarizes 148 experiments at various densities and flow rates. The mean firing rate (min⁻¹) is represented in color. Red regions display the highest firing rates (period of ~6 min). Sporadic firing occurs in the blue regions (see also fig. S5). White regions correspond to regimes of no spontaneous firing; white vertical lines indicate nonlinear breaks in the x axis. **(C)** Firing rate as a function of the ratio between cell density and flow rate (ρ/k) (blue circles). Mean $\pm$ SE (indicated in red) are computed from equi-populated bins. Four points around a value of -2 on the x axis were considered outliers because they were sampled at very low k (flow rates < 1 ml/min), where the effect of extracellular PDE is nonnegligible (30).

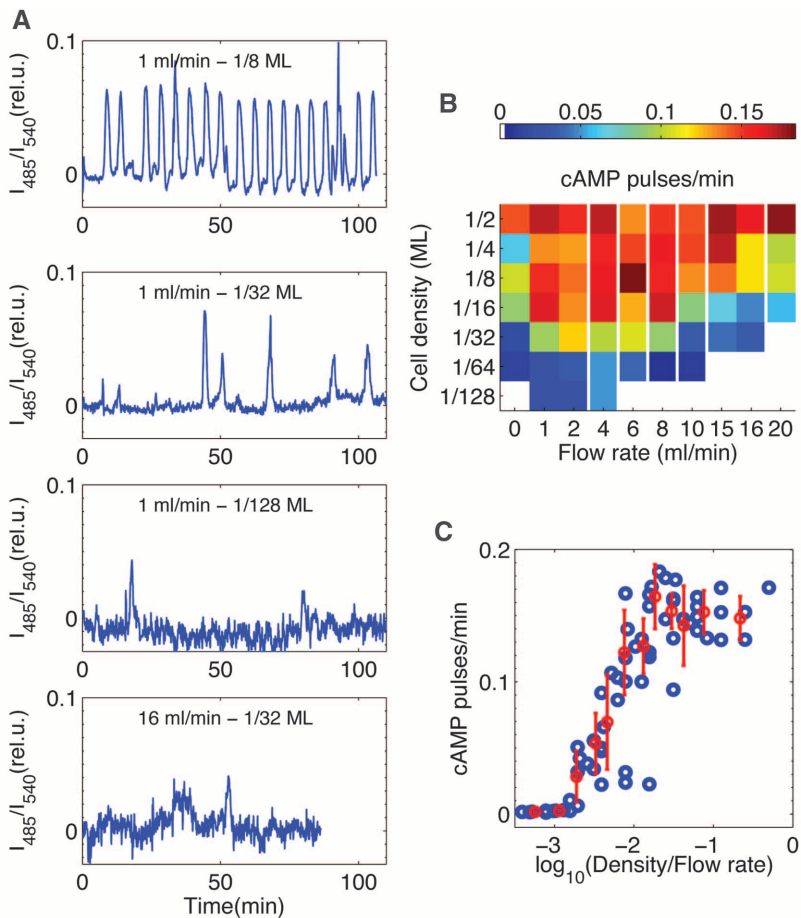
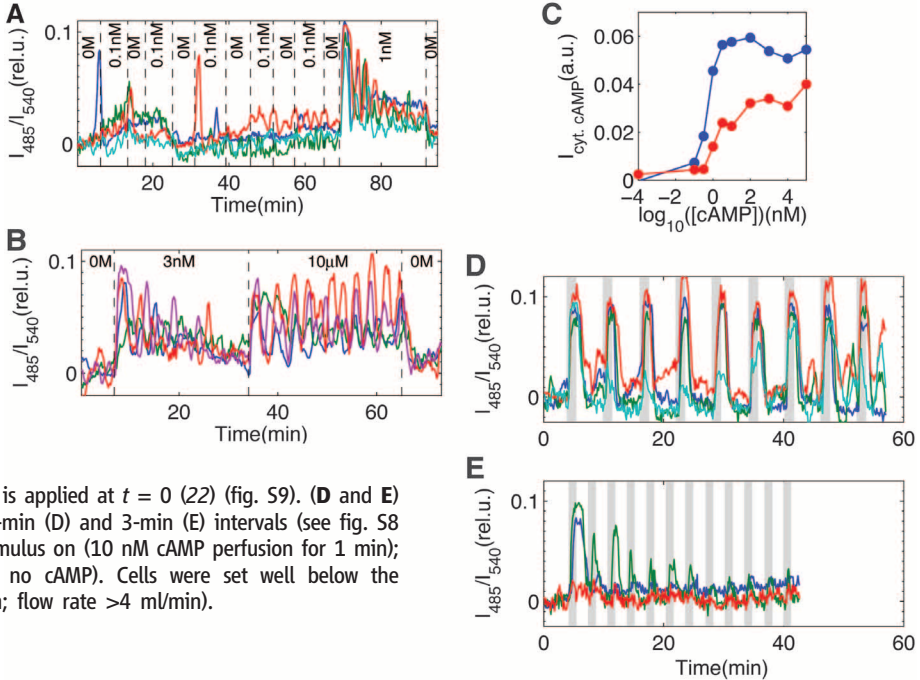


Fig. 3. The cAMP-induced cytosolic cAMP signaling response of isolated individual cells in a perfusion chamber. **(A)** Representative time courses of subthreshold response (four cells are represented by different colors). The concentration of extracellular cAMP was changed at time points indicated by dashed lines (0 M, perfusion with buffer only, no cAMP). **(B)** Damped and persistent oscillations of cytosolic cAMP when perfused with 3 nM and 10 μ M exogenous cAMP, respectively (colors indicate four individual cells). **(C)** Single-cell input/output relation of time- and cell-averaged responses to applied cAMP ranging from 100 pM to 100 μ M (blue and red lines correspond to 3- and 30-min averages, respectively). The relative cAMP output of an isolated cell for a given input stimulus over a time period T is evaluated here by computing the time-integral of I_{485}/I_{540} given by $I_{\text{cyt.cAMP}}(T) = \frac{1}{T} \int_0^T I_{485}(t)/I_{540}(t) dt$, where the stimulus is applied at $t = 0$ (22) (fig. S9). **(D and E)** Individual cells responding to repetitive stimuli at 6-min (D) and 3-min (E) intervals (see fig. S8 for 4-min and 15-min intervals). Shaded regions, stimulus on (10 nM cAMP perfusion for 1 min); white regions, stimulus off (buffer perfusion only, no cAMP). Cells were set well below the transition point shown in Fig. 2C (separation >1mm; flow rate >4 ml/min).



that guides aggregation of individual amoeboid cells. Most of our understanding of the cAMP oscillations in *Dictyostelium* is based on population-level optical-density observations (15–17) or isotope dilution assays of cAMP on fixed samples (14, 18–20). Because such measurements hide possible cell-cell heterogeneities, it is unknown whether the initiation of the periodic behavior is due to synchronization of cells that can autonomously oscillate regardless of interactions with other cells, or whether it is a “dynamical quorum sensing”-type phenomenon (3)—i.e., individual cells remain nonoscillatory unless the entire population becomes oscillatory.

To elucidate the onset of the cAMP oscillations, we used a fluorescence resonance energy transfer (FRET)-based sensor (21) to directly monitor cytosolic cAMP in live *Dictyostelium* cells (22) (fig. S3). Inhibiting synthesis of cAMP inhibited oscillations, confirming that changes in FRET efficiency reflect changes in cytosolic cAMP concentrations (22) (fig. S1). A representative time course of development is shown in Fig. 1, A and B (movie S1). Cells began to fire synchronously 5 hours after nutrient deprivation with pulses occurring sporadically every 15 to 30 min (Fig. 1B; 5 to 7 hours). Over the next 2 hours, the period of firing shortened to 8 min and thereafter to 6 min when cells began to aggregate. The entire population participated in the firing from the first pulse (Fig. 1C, leftmost panel). Differences in the phase of the oscillations depending on the regions indicate that pulses propagated in space as waves (fig. S7). Different spatial locations competed for wave initiation (Fig. 1C, second panel from the left). However, a single region eventually dominated and determined the aggregation center (Fig. 1A, middle panel). Tracking of

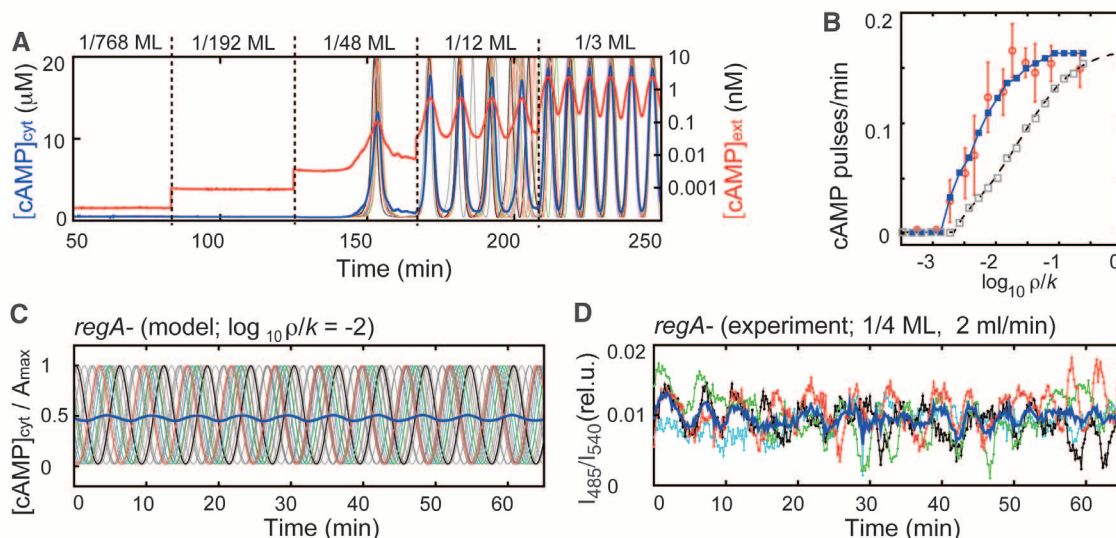
individual cells revealed a continuous exchange of cells to and from the signaling centers. Together, these observations suggest that the oscillations do not originate from autonomous activities of specialized cells, but rather that they probably result from inhomogeneous fluctuations in the concentration of extracellular signaling molecules that favor pulse generation.

To probe the extracellular conditions necessary to initiate periodic pulses, we placed cells in a perfusion chamber that provides rapid mixing and exchange of extracellular buffer to ensure a uniform and controlled environment. Pulsing rates were systematically measured for a wide range of cell densities and dilution rates. Representative data are shown in Fig. 2A. At sufficiently high densities ($\sim 10^3$ cells/mm²) and under moderate flow speed (~ 1 ml/min), cells periodically fire approximately once every 6 min on average (Fig. 2A, top panel), which is typically observed in an intact population near a monolayer density (14, 16, 17) (Fig. 1B). With decreasing cell density, the synchronized pulses become sporadic and finally cease (Fig. 2A). The dependence of the firing rate on cell density and dilution rate is presented as a phase diagram (Fig. 2B). The frequency of pulsing at flow rates >1 ml/min is proportional to a single parameter, ρ/k , which defines the ratio of cell density ρ to flow rate k (Fig. 2C). The pulsing rate is close to zero when ρ/k is small. As ρ/k is increased, the frequency increases logarithmically and levels off at ~ 6 -min oscillations.

To understand the origin of this dynamical transition, we quantified the response of single isolated cells to continuous application of exogenous cAMP (Fig. 3, A and B). In the absence of extracellular cAMP, cytosolic cAMP concen-

trations remain steady with some fluctuations (fig. S4). At subnanomolar stimulation, cells pulse randomly (Fig. 3A). Upon application of nanomolar concentrations of cAMP, cells display excitatory behavior (1 nM in Fig. 3A and 3 nM in Fig. 3B)—a sharp transient rise that peaks after ~ 30 s is followed by smaller peaks that gradually attenuate. Damping of the response becomes less marked at micromolar concentrations of cAMP (Fig. 3B; 10 μ M). The oscillatory responses persist at 2.5- to 8-min periods, which vary from cell to cell. The peaks appear even at 100 μ M cAMP (fig. S9), well above the saturation dose of the cAMP receptors (23), suggesting that cytosolic oscillations can occur without periodic changes in the concentration of extracellular cAMP. However, this persistent oscillatory behavior depends strictly on the presence of extracellular cAMP, because when the stimulus is removed, cytosolic cAMP returns to its prestimulus level within 30 to 60 s (Fig. 3A at 90 min; Fig. 3B at 65 min). From fluorometric measurements, we estimate that the basal and the peak concentrations of cytosolic cAMP correspond on average to ~ 400 nM and ~ 10 μ M, respectively (fig. S3). Figure 3C summarizes the cytosolic cAMP responses in the form of an input/output relation (see also fig. S9). The input corresponds to the applied cAMP concentrations and the output to the integrated response over two time intervals: (i) 3 min, corresponding to the initial peak of the response (Fig. 3C; blue curve); and (ii) 30 min (red curve). The 3-min interval exhibits a half-maximal output at 0.5 ± 0.1 nM cAMP, suggesting that a cell can be excited by a very low number of cAMP molecules. For the longer interval, the half-maximal concentration is 1.7 ± 0.2 nM owing to damping of the response.

Fig. 4. Dynamical quorum sensing-type transition in a population of excitable/oscillatory switch elements. (A) Representative simulated time courses of average cAMP concentrations (cytosolic $[cAMP]_{\text{cyt}}$ and extracellular $[cAMP]_{\text{ext}}$ in linear and logarithmic scales, respectively). Thin colored lines indicate $[cAMP]_{\text{cyt}}$ in individual cells. Cell densities were increased in incremental steps of 1/768 ML, 1/192 ML, 1/48 ML, 1/12 ML, and 1/3 ML at $k = 5$ ml/min. (B) Firing rate as a function of ρ/k as predicted by the coupled phase model with (blue line; eq. S5) or without (gray squares; eq. S5) incorporation of stochastic pulsing. For comparison, see experimental data from Fig. 2C (red circles) and mean-field approximation (dashed black line; eq. S6). (C) Model prediction of desynchronization at elevated cytosolic cAMP concentrations [a 10-fold increase in maximum (A_{max}) and basal (A_{bas}) cytosolic cAMP concentrations; eq. S1]. $[cAMP]_{\text{cyt}}$ normalized by A_{max} in individual cells (thin



gray lines; four cells are highlighted by colors). Blue line indicates population average. (D) Poorly synchronized population-level oscillations in *regA*[−] mutants whose degradation of intracellular cAMP is reduced (1/4 ML; 2 ml/min). Colors indicate average signals by cells in four 100 μ m by 100 μ m regions separated by ~ 150 μ m. Blue line indicates average of the four regions.

The results in Figs. 2 and 3 indicate that accumulation of extracellular cAMP ($[cAMP]_{ext}$) in the population is essential for excitatory and oscillatory cell responses. At low p/k , if cells secrete cAMP on average at a constant rate r , then $[cAMP]_{ext}$ changes according to

$$\frac{d[cAMP]_{ext}}{dt} = \rho r - \frac{k}{V_T} [cAMP] \quad (1)$$

which states that variations in cell density ρ alter the effective synthesis rate of extracellular cAMP, while the rate of dilution k divided by the chamber volume V_T determines how fast extracellular cAMP is removed. Assuming that the system is at steady state in this regime, $[cAMP]_{ext}$ must be proportional to the parameter ρ/k (eq. S4), and hence Eq. 1 assumes a constant cell secretion rate for cAMP. Isolated cells exposed to extracellular cAMP below the threshold concentration of ~ 500 pM (Fig. 3C), however, display nonnegligible excitable dynamics that appear to be stochastic (Fig. 3A and fig. S4). This observation matches well with the sporadic pulses observed experimentally at the population level (fig. S5), when p/k is near the transition point (i.e., $p/k \sim 10^{-3}$ in Fig. 2C). Incorporating first-order secretion (24) into Eq. 1, we computed the effective synthesis rate per monolayer cell density to be $r = 5.2$ nM/min (by eqs. S3 and S8), implying that near the transition point, cells should only be able to accumulate extracellular cAMP of less than ~ 10 pM (eq. S7). Such low concentrations, however, are more than an order of magnitude below the responsive range of a single cell (Fig. 3C), indicating a limitation of the static picture provided by Eq. 1.

At high p/k , the firing rate reaches a plateau ($\sim 1/6$ min $^{-1}$ in Fig. 2C), which cannot be deduced from the observed cell responses to persistent stimuli (Fig. 3C) alone. When isolated cells are repetitively stimulated with 10 nM cAMP at 6-min intervals, cells responded in pace with the stimuli (Fig. 3D). However, at shorter intervals the response gradually diminished (Fig. 3E and fig. S8A), and at longer intervals random bursts appeared spontaneously (fig. S8B). Thus, an individual *Dictyostelium* cell behaves as a resonance filter that selects a stimulus at 6-min intervals. That is, extracellular cAMP must be removed for a certain time period for cells to respond to further stimuli. Such a time scale is a single-cell-level property. The requirement of cAMP removal is consistent with population experiments that we performed in the absence of flow (< 1 ml/min; Fig. 2B). They demonstrate that synchronized oscillations require removal of extracellular cAMP by extracellular phosphodiesterase (PDE, the enzyme that degrades extracellular cAMP) (figs. S2 and S10A). Together with the observed cytosolic oscillations in cells under prolonged stimulation (Fig. 3B), these observations indicate that in addition to the time it takes for the extracellular cAMP to return to its original prepulse concentration (a global process mediated by extracellular PDE), there exist local mechanisms that monitor time intracel-

lularly before individual cells fully recover their state of excitability.

To determine quantitatively the origin of synchronized pulses in the population, we incorporated the observed response behavior into Eq. 1. To this end, the single-cell-level dynamics of cytosolic cAMP is described by a phase equation (eqs. S1 and S2 and fig. S6A) that reproduces the pulsatile cAMP output observed in Fig. 3 (fig. S6B). When such cellular elements are coupled (eq. S5), the population switches from a quiescent to an oscillatory state at a critical cell density (Fig. 4A). The model can accurately reproduce the frequencies of the synchronized oscillations (Fig. 4B; blue line and red circles) only when the random pulsing observed at subnanomolar concentrations (Fig. 3A and fig. S4) is taken into account. Without random pulsing, the curve appears slanted and shifts toward higher p/k (Fig. 4B; gray line). Hence, our model confirms the importance of sporadic random firing at the onset of synchronized pulses in the population, as observed experimentally in fig. S5. At subnanomolar concentrations of extracellular cAMP, the probability that a cell is randomly excited increases (Fig. 3A and fig. S4), enhancing the chance for other cells to fire (fig. S6C). A chain reaction of excitation is thus generated that can give rise to synchronized pulses [Fig. 4A (1/48 ML) and fig. S6C] even at subnanomolar concentrations below the threshold. Thus, the question of whether specialized cells initiate synchronized pulses is ill posed; the initiation process is inherently collective and stochastic.

Synthesis of intracellular cAMP requires a receptor-mediated activation of adenylyl cyclase ACA (fig. S1, D to G)—the main enzyme responsible for converting adenosine 5'-triphosphate (ATP) to cAMP during this stage of *Dictyostelium* development. Binding of cAMP to the membrane-bound receptor induces production of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) that recruits an activating factor containing a specific PIP₃-binding domain [pleckstrin homology (PH) domain] to the plasma membrane (25). Given the small number of extracellular cAMP molecules necessary to elicit a response in cytosolic cAMP (Fig. 3C), the stochastic nature of the pulsing may originate from random binding of cAMP to the membrane-bound cAMP receptor. However, the threshold value in the response (0.5 ± 0.1 nM in Fig. 3C; blue curve) is an order of magnitude below the affinity of the receptor (23). Because a similar dosage dependence of membrane translocation of a PH domain-containing protein has been observed (26), our input/output relation (Fig. 3C, blue curve) most likely reflects the kinetics downstream of the membrane receptors but upstream of PIP₃.

That cells can be oscillatory even at the single-cell level in the perfusion setup (Fig. 3, B, D, and E) might indicate that the emergence of synchronized oscillations in the population is not a collective phenomenon. However, these cell-intrinsic oscillations appear only when the

concentrations of extracellular cAMP are kept above 1 nM (Fig. 3B and fig. S9). Before extracellular cAMP can rise to this level spontaneously in a cell population, synchronized pulses must emerge. In our perfusion experiments, at low p/k the population remains in a state in which pulses are emitted sporadically. In contrast, when extracellular cAMP is increased to a sufficiently high level, cells become transiently oscillatory. This occurs at high p/k where the pulsing frequency reaches a plateau (Fig. 2C). At that level, pulse timing is no longer dictated by the occurrence of random pulses in the population, but rather by an oscillatory mechanism at the single-cell level that gates and paces the timing. As a result, the pulses appear regularly at ~ 6 -min intervals (Fig. 2A). One would expect that disruption of intracellular PDE (RegA) (27) should have a strong deleterious effect if these cell-intrinsic oscillatory dynamics involve a feedback regulation via cytosolic cAMP (20). However, if the main effect of disrupting RegA is to increase the overall concentration of cAMP secreted into extracellular space, *regA*⁻ cell populations should still be able to oscillate albeit with only partial synchrony (Fig. 4C) because the extracellular cAMP concentration should almost saturate the dynamic range of the input/output relation (Fig. 3C and fig. S6B). In agreement with this model prediction, we observe that *regA*⁻ cells oscillate but are poorly synchronized (Fig. 4D). Furthermore, because intrinsic oscillations observed at saturating dose of extracellular cAMP are also present in isolated *regA*⁻ cells (fig. S10B), the oscillations at the single-cell level do not require a RegA-mediated feedback loop.

Finally, the entire sequence of events in an intact cell population (Fig. 1), from the onset of sporadic firing to determination of the final periodic signaling center, can be inferred from the perfusion experiments. The key enzymes that determine the rates of secretion (r) and of cAMP decay (k) in Eq. 1 are ACA and extracellular PDE, respectively (figs. S1, S2, and S10A), both of which are expressed at very low levels but are induced during the first 4 hours after starvation (13, 28). When cAMP slowly begins to accumulate in the extracellular space, initial pulses are randomly elicited owing to the stochastic sub-threshold dynamics at the single-cell level. Although several centers compete for dominance, the results in Figs. 2C and 4B predict that sites that accumulate more extracellular cAMP than others pulse at a higher frequency. Such sites will entrain other sites that fire less often (10) and therefore survive. Thus, the first site that accumulates enough extracellular cAMP to support the saturating frequency (~ 6 -min period) becomes the persistent oscillatory center. This not only allows aggregation of more cells to the same location, but may also facilitate later development because cAMP is required for cell differentiation (29).

The signaling centers in *D. discoideum* are dynamic entities that self-organize in the population. The onset of synchronized pulses occurs

by a switchlike response of individual cells to an external threshold concentration. The initiation is highly dynamic and collective, because the threshold and the frequency of the response cannot be attributed solely to those at the single-cell level. As random cells continue to emit pulses sporadically, extracellular cAMP accumulates so that, at the peak of the synchronized pulses, cells become transiently oscillatory. The combination of these two strategies, one global and the other local, may allow cells to first determine the global maximum of extracellular cAMP concentration and then aggregate as the pulses become self-sustainable and are periodically emitted from such locations.

References and Notes

1. C. M. Waters, B. L. Bassler, *Annu. Rev. Cell Dev. Biol.* **21**, 319 (2005).
2. A. F. Taylor, M. R. Tinsley, F. Wang, Z. Huang, K. Showalter, *Science* **323**, 614 (2009).
3. S. De Monte, F. d'Ovidio, S. Danø, P. G. Sørensen, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 18377 (2007).
4. T. Danino, O. Mondragón-Palmino, L. Tsimring, J. Hasty, *Nature* **463**, 326 (2010).
5. J. Buck, E. Buck, *Science* **159**, 1319 (1968).
6. S. Yamaguchi *et al.*, *Science* **302**, 1408 (2003).
7. I. H. Riedel-Kruse, C. Müller, A. C. Oates, *Science* **317**, 1911 (2007).
8. K. Wiesenfeld, P. Colet, S. H. Strogatz, *Phys. Rev. Lett.* **76**, 404 (1996).
9. I. Z. Kiss, Y. M. Zhai, J. L. Hudson, *Science* **296**, 1676 (2002).
10. A. T. Winfree, in *The Geometry of Biological Time (Biomathematics Series)* (Springer-Verlag, New York, 1980).
11. D. McMillen, N. Kopell, J. Hasty, J. J. Collins, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 679 (2002).
12. J. Garcia-Ojalvo, M. B. Elowitz, S. H. Strogatz, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 10955 (2004).
13. A. Goldbeter, *Biochemical Oscillations and Cellular Rhythms. The Molecular Bases of Periodic and Chaotic Behaviour* (Cambridge Univ. Press, Cambridge, 1996).
14. K. J. Tomchik, P. N. Devreotes, *Science* **212**, 443 (1981).
15. J. D. Gross, M. J. Peacey, D. J. Trevan, *J. Cell Sci.* **22**, 645 (1976).
16. F. Siegert, C. Weijer, *J. Cell Sci.* **93**, 325 (1989).
17. S. Sawai, P. A. Thomason, E. C. Cox, *Nature* **433**, 323 (2005).
18. G. Gerisch, U. Wick, *Biochem. Biophys. Res. Commun.* **65**, 364 (1975).
19. P. N. Devreotes, P. L. Derstine, T. L. Steck, *J. Cell Biol.* **80**, 291 (1979).
20. M. Maeda *et al.*, *Science* **304**, 875 (2004).
21. V. O. Nikolaev, M. Bünemann, L. Hein, A. Hannawacker, M. J. Lohse, *J. Biol. Chem.* **279**, 37215 (2004).
22. Materials and Methods are available as supporting material on Science Online.
23. R. L. Johnson *et al.*, *J. Biol. Chem.* **267**, 4600 (1992).
24. M. C. Dinauer, S. A. MacKay, P. N. Devreotes, *J. Cell Biol.* **86**, 537 (1980).
25. F. I. Comer, C. K. Lippincott, J. J. Masbad, C. A. Parent, *Curr. Biol.* **15**, 134 (2005).
26. L. Bosgraaf, I. Keizer-Gunnink, P. J. Van Haastert, *J. Cell Sci.* **121**, 3589 (2008).
27. P. A. Thomason *et al.*, *EMBO J.* **17**, 2838 (1998).
28. N. Iranfar, D. Fuller, W. F. Loomis, *Eukaryot. Cell* **2**, 664 (2003).
29. L. Kim, A. Harwood, A. R. Kimmel, *Dev. Cell* **3**, 523 (2002).
30. At very low k (flow rates $< 1\text{ ml/min}$), the effect of extracellular PDE is nonnegligible. Pulses are no longer observed when dithiothreitol is applied to inhibit extracellular PDE (fig. S2), demonstrating that periodic firing under these conditions depends on degradation of extracellular cAMP by extracellular PDE released by the cells. At higher k , clearing of extracellular cAMP from the medium is mainly determined by dilution.
31. We thank V. O. Nikolaev and M. J. Lohse for providing vector containing Epac1-camps; M. Kitahara and E. Hada for technical help, E. C. Cox, S. Ishihara, K. Kaneko, H. Kori, and J. Teramae for discussions and suggestions; and the Japan Society for the Promotion of Science (JSPS) postdoctoral fellowship (T.G.), K. Kaneko, and the ERATO-JST for support.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1183415/DC1
Materials and Methods
Figs. S1 to S10
References
Movie S1

15 October 2009; accepted 19 March 2010
Published online 22 April 2010;
10.1126/science.1183415
Include this information when citing this paper.

Structural Insights into the Assembly and Function of the SAGA Deubiquitinating Module

Nadine L. Samara,^{1*} Ajit B. Datta,^{1,2*} Christopher E. Berndsen,^{1,2} Xiangbin Zhang,^{1,2} Tingting Yao,³ Robert E. Cohen,³ Cynthia Wolberger^{1,2†}

SAGA is a transcriptional coactivator complex that is conserved across eukaryotes and performs multiple functions during transcriptional activation and elongation. One role is deubiquitination of histone H2B, and this activity resides in a distinct subcomplex called the deubiquitinating module (DUBm), which contains the ubiquitin-specific protease Ubp8, bound to Sgf11, Sus1, and Sgf73. The deubiquitinating activity depends on the presence of all four DUBm proteins. We report here the 1.90 angstrom resolution crystal structure of the DUBm bound to ubiquitin aldehyde, as well as the 2.45 angstrom resolution structure of the uncomplexed DUBm. The structure reveals an arrangement of protein domains that gives rise to a highly interconnected complex, which is stabilized by eight structural zinc atoms that are critical for enzymatic activity. The structure suggests a model for how interactions with the other DUBm proteins activate Ubp8 and allows us to speculate about how the DUBm binds to monoubiquitinated histone H2B in nucleosomes.

The covalent modification of chromatin is a core feature of eukaryotic transcription (1). The SAGA complex regulates genes transcribed by RNA polymerase II by carrying out multiple functions that include histone acetylation and deubiquitination (2, 3). The 1.8-megadalton SAGA complex, which contains 21 proteins conserved from yeast to humans (4), plays a role in transcription activation and also couples transcription elongation with export of the nascent RNA through the nuclear pore complex (5). SAGA is recruited to promoter regions

by activator proteins, where it catalyzes the cleavage of monoubiquitin from lysine-123 (K123) of histone H2B, as well as acetylation of histone H3 (6). Both of these activities are thought to be important for evicting nucleosomes from the promoter region and for facilitating transcription elongation (7). The yeast SAGA complex has been a model for studying SAGA function in the transcription cycle. Histone acetylation by SAGA is mediated by the Gcn5 histone acetyltransferase subunit, whereas histone H2B deubiquitination is mediated by a discrete

subcomplex known as the deubiquitinating module (DUBm) (3, 8). The yeast DUBm comprises four proteins: the ubiquitin-specific protease (Ubp8) ubiquitin hydrolase, Sgf11, Sus1, and Sgf73 (9, 10). Sgf73 tethers DUBm to the rest of the SAGA complex through a central domain; the N-terminal domain forms an integral part of the DUBm (9). Sgf73, along with Sus1, also facilitates SAGA's role in nuclear export by binding to components of the nuclear pore complex (11). Although Ubp8 contains a ubiquitin-specific hydrolase (Usp) domain (12), the protein is inactive unless it is in complex with the other three DUBm proteins (9, 13). The structural basis for Ubp8 activation is not understood, nor is its dependence on the other three DUBm proteins to form a stable complex that can specifically target H2B for deubiquitination.

Drosophila and human SAGA include a DUBm that functions analogously to the yeast DUBm and comprises homologs of the yeast proteins (10, 14, 15). In addition, the human DUBm, consisting of USP22, ATXN7L3, ENY2 and ATXN7 (homologs of Ubp8, Sgf11, Sus1, and

¹Department of Biophysics and Biophysical Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. ²Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. ³Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: wolberg@cjhmi.edu

Sgf73, respectively), also plays a role in telomere maintenance by regulating the stability of TRF1, a component of the telomeric shelterin complex (15). These additional functions suggest that the human DUBm may have other substrates that remain to be identified. The importance of DUBm function in humans is highlighted by the observations that USP22 is a cancer stem cell marker (16) and that ATXN7 is the affected protein in the polyglutamine expansion disease, spinocerebellar ataxia type 7 (17, 18).

In order to gain insight into the basis for DUBm assembly, activity, and substrate binding, we determined the structure of the heterotetrameric DUBm with and without bound ubiquitin aldehyde (Ubal), an inhibitor that forms a reversible covalent bond with the active-site cysteine (19, 20). Soluble, enzymatically active complex was obtained by coexpressing the intact Ubp8 (4741 amino acids), Sgf11 (99 amino acids), and Sus1 (96 amino acids) proteins along with an N-terminal fragment of Sgf73 (residues 1 to 96) that is sufficient for enzymatic activity (9). The coexpressed DUBm used in the structural study cleaves ubiquitin C-terminal 7-amido-4-methylcoumarin (ubiquitin-AMC) with an enzymatic rate (k_{cat}) of 0.17 s^{-1} and a Michaelis constant (K_m) of $1.5 \text{ }\mu\text{M}$, giving a k_{cat}/K_m value of $1.1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (fig. S1). The activity is inhibited by Ubal with an apparent dissociation constant (K_i) of $0.2 \text{ }\mu\text{M}$ (fig. S2). The coexpressed DUBm can also cleave ubiquitin from the free histone substrates, H2B and H2A (fig. S3), which demonstrates that the complex retains the reported deubiquitinating activity of the complete SAGA complex. The crystal structure of the DUBm bound to Ubal was determined by selenomethionine multiwavelength anomalous dispersion phasing and refined at $1.90 \text{ }\text{\AA}$ resolution to an R/R_{free} of $15.5/20.3\%$. The $2.45 \text{ }\text{\AA}$ resolution structure of free DUBm was determined by molecular replacement with the high-resolution DUBm structure as a search model, and refined to an R/R_{free} of $20.4/28.1\%$. Data collection and refinement statistics are shown in table S1. Sequence alignments for all four DUBm proteins, annotated with the positions of secondary structural elements, are shown in fig. S4. The description of the structure that follows focuses on the higher-resolution model of the Ubal-bound complex, except where noted.

The structure of the DUBm shows Ubp8, Sgf11, Sus1, and Sgf73 to be remarkably intertwined (Fig. 1, A and B). Each polypeptide contacts the other three proteins in the complex, which explains why active complex formation requires the presence of all four DUBm subunits (9, 13). With the exception of Ubp8, which contains two globular domains separated by a short linker, the conformations of the other three proteins are largely dependent on their interactions with other DUBm proteins. The complex is organized into two lobes, each containing one of the two domains of Ubp8: the N-terminal zinc finger-ubiquitin binding protein (ZnF-UBP)

domain and the C-terminal enzymatic Usp domain (Fig. 1, A and B). The ZnF-UBP lobe (Fig. 1C) is organized around the long N-terminal helix of Sgf11, which binds the Ubp8 ZnF-UBP domain on one face and Sus1 on the opposing face. The 40 N-terminal residues of Sgf73 comprise the remainder of this lobe and primarily contact the Ubp8 ZnF-UBP domain and Sus1 with short helices (Fig. 1, A and C). The second lobe of DUBm contains the ~ 350 -amino acid Usp domain of Ubp8 (Fig. 1, A and D). Ubiquitin aldehyde binds, as expected, to the “fingers” region of the Usp domain (21), which consists of an antiparallel β sheet and a zinc-binding domain (Fig. 1D), and forms a covalent bond between the modified C-terminal glycine and the active-site cysteine. To the opposite side of the active site lies the N-terminal zinc finger domain of Sgf11, which binds to the face of the Usp domain (Fig. 1D). An extended well-ordered linker region joins the Sgf11 zinc finger with its

N-terminal helix (Fig. 1B) and forms a bridge between the two lobes of the DUBm while forming additional contacts with Ubp8. The interactions at the interface between the two lobes are mediated primarily by 50 C-terminal residues of the Sgf73 fragment (45 to 95), which are buried between the two lobes. This portion of Sgf73 contains a short α helix and a zinc finger domain that are connected by 20 residues that lack secondary structure, yet are well ordered and have low temperature factors. Sus1 helices $\alpha 2$ and $\alpha 3$ mediate additional interface contacts with Ubp8 on the back face of the Usp domain. Together, Sgf11, Sus1, and Sgf73 bury a total of $7910 \text{ }\text{\AA}^2$ of the Ubp8 surface. The overall features of the DUBm complex are preserved in the structure of apo DUBm, which crystallizes with different crystal packing. This indicates that the unusual arrangement of proteins in the DUBm complex is not an artifact of crystal contacts.

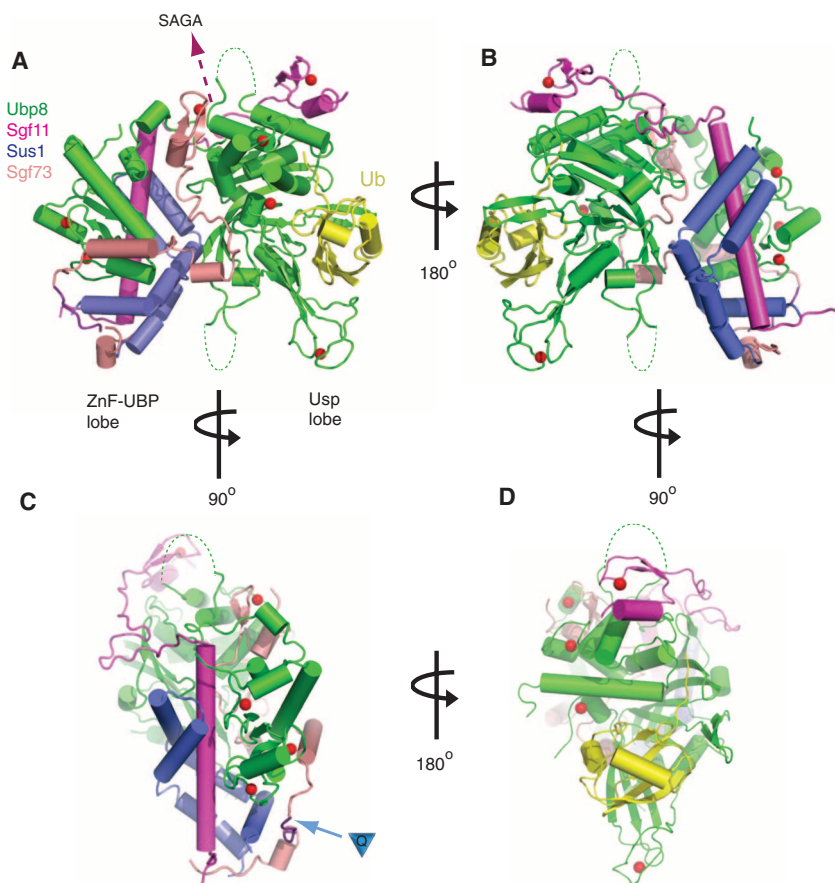


Fig. 1. Overall structure of the SAGA DUBm. (A) View of DUBm bound to ubiquitin aldehyde (Ubal). The ZnF-UBP lobe and Usp lobe are labeled. The DUBm protein coloring scheme is shown at left; Ubal is yellow, with zinc atoms shown as red spheres. Disordered loop residues are indicated with dotted lines. Residues in Sgf73 that are ordered in the structure of the apo DUBm are shown in purple. (B) View of DUBm rotated 180° about the vertical as compared with (A). (C) View of the ZnF-UBP lobe. The Ubp8 ZnF-UBP domain and Sus1 bind on opposite faces of the Sgf11 N-terminal helix. The Sgf73 residues shown in purple are disordered in the Ubal-bound complex but are ordered in the apo complex. The insertion point for polyglutamine expansion in the human homolog, ATXN7, is indicated by an inverted triangle containing the letter, Q. (D) View of the Usp lobe. The fingers region to which ubiquitin (yellow) binds is at the bottom and the Sgf11 zinc finger (magenta) is at the top.

The binding of Sgf73 between the two lobes of the DUBm is consistent with its role in anchoring the DUBm to the rest of the SAGA complex (9, 10) and in helping to activate DUB activity. As compared with Sgf11 and Sus1, which are also nonglobular, Sgf73(1–96) appears to be the most dependent on other DUBm proteins for its observed conformation, because it contains the fewest intrachain contacts. In the intact protein, the remaining C-terminal residues of Sgf73 link the DUBm to the

SAGA complex (Fig. 1A), with residues 227 to 402 mediating interactions with the other SAGA proteins (9, 10). There is a low-complexity acidic region (residues 130 to 162) between the SAGA-binding and DUBm-binding regions of Sgf73, which suggests that the DUBm might be flexibly tethered to the remainder of the SAGA complex. Sgf73(1–96) contains a zinc finger that is coordinated by two cysteines (C78, C81), and a histidine (H93) (6). The fourth ligand in the DUBm-Ubal complex is an aspartic acid (D95)

that is conserved in the human homolog, ATXN7 (fig. S4); however, this residue does not coordinate the zinc in the apo DUBm structure and, instead, is part of an extended C-terminal helix. It is likely that, in the full-length protein, the histidine (H97) or cysteine (C98) residues that are missing from the crystallized fragment serve as the fourth zinc-coordinating ligand.

The incorporation of Sgf73 into the DUBm depends on the integrity of the zinc finger, because a slightly shorter fragment containing residues 1 to 92 is defective in binding to the other DUBm subunits (9). We therefore tested the effect of zinc chelation on the incorporation of Sgf73 and the other DUBm subunits into the complex. In the absence of EDTA, the DUBm sediments with an $S_{20,w}$ value of 4.9 (Fig. 2), as expected for a single globular 90-kD species with a somewhat asymmetric axial ratio. The addition of 2 mM EDTA causes the complex to dissociate into two principal species corresponding to Sgf73 and the Ubp8-Sgf11-Sus1 heterotrimer (Fig. 2). We note that the Sgf11-Sus1 heterodimer sediments as multiple species (fig. S5) that do not account for any of the observed peaks. On the basis of the DUBm structure, the stable association between Ubp8, Sgf11, and Sus1 is most likely maintained by contacts between the long N-terminal helix of Sgf11, Sus1, and the ZnF-UBP domain. The loss of complex integrity is accompanied by a loss of activity, which is observed upon addition of increasing concentrations of EDTA, as well as increasing incubation time (fig. S6). In addition to dissociating Sgf73, which is known to compromise DUBm activity (10), it is also possible that the EDTA further affects enzymatic activity by chelating the zinc atoms in the enzymatic domain of Ubp8 (Fig. 3, A and B). The importance of wild-type Sgf73 function is highlighted by the effect of polyglutamine expansions in the N terminus of the human homolog, ATXN7 (18). These insertions of up to 300 glutamine residues compromise SAGA activity and give rise to defects in transcription (22). The polyglutamine expansion occurs near the N terminus of Sgf73 (18), in the coil region between the first two helices (Fig. 1C). It is possible that large insertions in this region disrupt Sgf73 association with the other DUBm proteins, which compromises enzymatic activity or causes them to dissociate from the SAGA complex. Alternatively, polyglutamine tracts may cause Sgf73 to aggregate and, therefore, to fail to bind to the other DUBm proteins.

The structures of the individual DUBm subunits contain some unexpected features. The overall fold of the Ubp8 catalytic domain is very similar to that of other Usp proteins (12). However, the catalytic domain of Ubp8 contains two bound structural zinc atoms (Fig. 3, A and B) that have not been previously observed (12). One of the bound zinc atoms (Fig. 3A) is located between helices α_9 and α_{10} (see fig. S4 for numbering) and is coordinated by H170, C174,

Fig. 2. Dissociation of the Sgf73 fragment from the DUBm complex. Velocity sedimentation of the DUBm in the presence (blue) and absence (black) of 2 mM EDTA, superimposed with the sedimentation of Sgf73(1–106) alone.

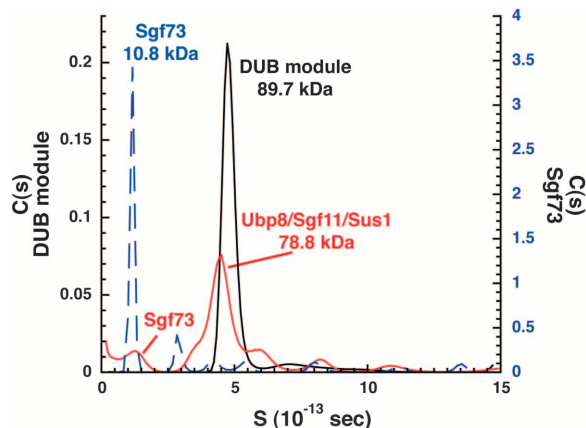
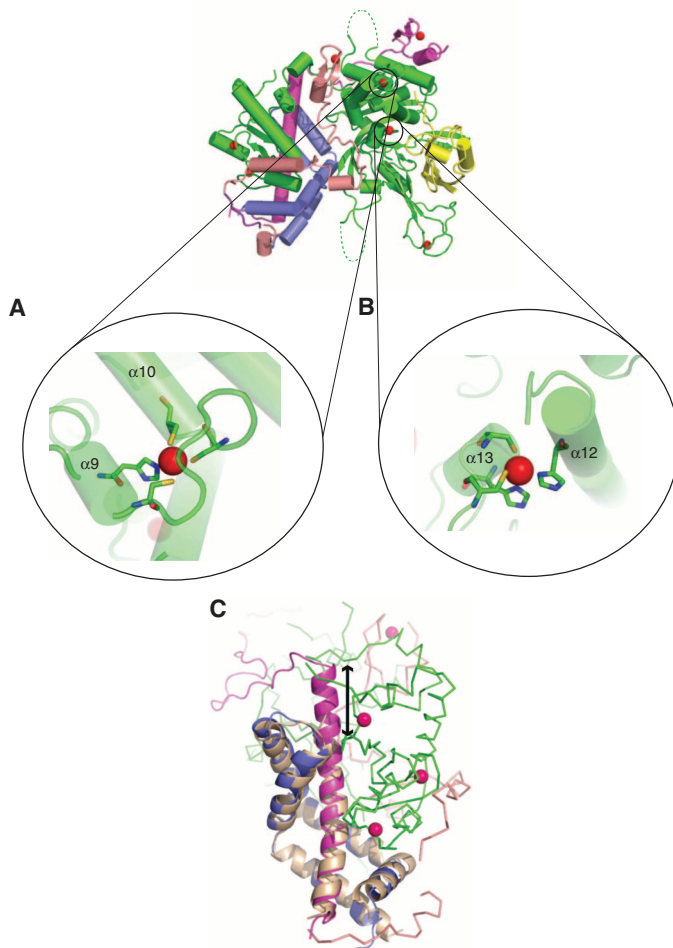


Fig. 3. Structural details of the DUBm. (A) Structural zinc coordination in the Ubp8 Usp domain located between α_9 and α_{10} . (B) Structural zinc coordination in the Ubp8 Usp domain located between α_{12} and α_{13} . (C) Superposition of the structure of Sus1/Sgf11(1–33) (copper) (PDB ID 3KIK) with the corresponding residues of Sus1 (blue) and Sgf11 (magenta) in the DUBm. The arrow indicates the additional helical residues in Sgf11 seen in the DUBm structure.



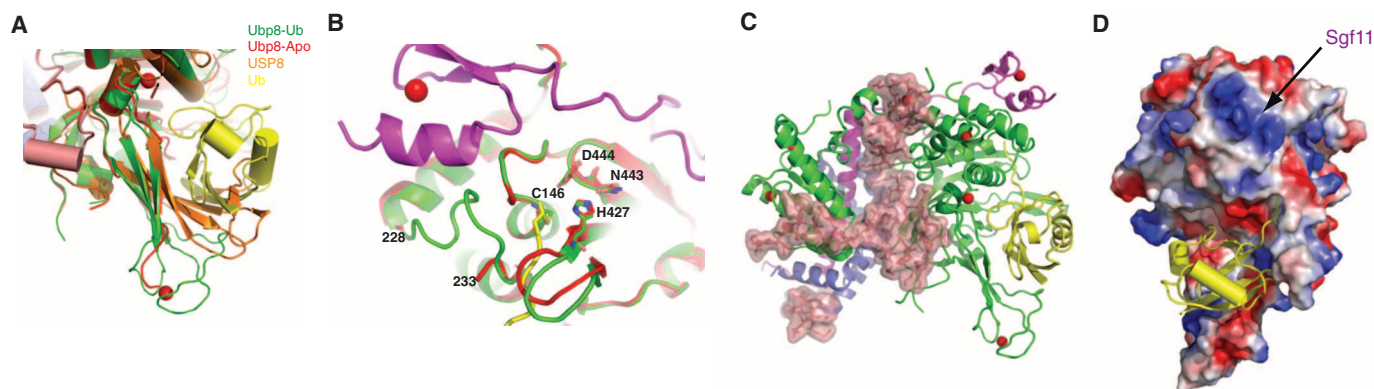


Fig. 4. Interaction between the DUBm and substrate. **(A)** Alignment of apo Ubp8, Ubp8-Ubal, and USP8. In the apo USP8 structure, the fingers region is collapsed and cannot accommodate ubiquitin without a conformational change. **(B)** Structural changes in the region of the Ubp8 active site. Residues 228 to 233 of the Ubp8 apoenzyme (red) are disordered in the absence of ubiquitin and a loop, 421 to 426, shifts into the groove where the C-terminal tail of ubiquitin (yellow) would bind. A second loop, which contains the active-site cysteine, C146,

maintains the same conformation in the presence and absence of ubiquitin and is stabilized by contacts with the Sgf11 zinc finger (magenta). The other active-site residues, H427, N443, and D444, also remain in the same orientation in both structures. **(C)** Structure of the DUBm showing a surface representation of Sgf73. The color scheme used is the same as in Fig. 1. **(D)** Electrostatic surface potential of the ubiquitin-binding face of the DUBm. The region of strong electropositive surface potential (blue) is due to the Sgf11 zinc finger.

C182, and C185, whereas the second bound zinc (Fig. 3B) is located in a nonconserved loop region between helices α 12 and α 13 and is coordinated by H250, C271, C273, and H276. Although these bound zinc atoms are not conserved among the vast majority of USP enzymes, sequence comparisons indicate that both of these sites are conserved in the Ubp8 homologs, human USP22 (fig. S4) and *Drosophila* Nonstop, which are SAGA DUBm subunits (14, 15). Another interesting feature of Ubp8 is that the ZnF-UBP domain—which, in other USP enzymes such as Usp5, binds ubiquitin (23)—has its ubiquitin-binding face occluded in the DUBm structure. That region of the domain, which is centered around β strands 2 to 5 (fig. S4), is largely buried at the interface with Sgf11. Although a role for ubiquitin binding to this domain cannot be ruled out when Ubp8 is not part of the observed complex, the absence of conserved ubiquitin-binding residues in Ubp8—as well as in the human homolog, USP22 (24)—makes this unlikely. Other ZnF-UBP domains that similarly lack conservation in the ubiquitin-binding residues and do not bind ubiquitin, such as in USP33 (25), may also play structural roles in those enzymes.

A structure of Sus1 that was previously determined in complex with residues 1 to 33 of Sgf11 (26) is virtually superimposable with the corresponding residues of Sgf11 and Sus1 in the DUBm complex (Fig. 3C). In the DUBm structure, however, which contains the intact 96-amino acid Sgf11 protein, the N-terminal Sgf11 helix is extended by an additional 10 amino acids at its C terminus; this presumably is due to the additional contacts with the Ubp8 ZnF-UBP domain (Figs. 1C and 3C). The ZnF-UBP domain also contacts helix α 1 of Sus1 and likely helps to stabilize its interaction with Sgf11. In the structure of Sus1 bound to Sgf11(1–33), helix

α 1 of Sus1 adopts widely variable orientations in the four complexes found in the asymmetric unit (26), only one of which contacts Sgf11 and matches the closed conformation observed in the DUBm. The closed conformation of Sus1 is also observed when it binds to Sac3 as part of the TREX-2 complex, which functions in mRNA export (27). Thus, the N-terminal helix of Sgf11 plays a scaffolding role similar to that of Sac3, which also forms a long α helix to which multiple proteins bind.

A comparison of the ubiquitin aldehyde (Ubal) DUBm complex with the apo DUBm complex reveals that a number of structural changes occur in Ubp8 when ubiquitin binds to the DUBm. The overall features of ubiquitin binding, including the arrangement of the catalytic residues, C146, H427, N443, and D444 (see fig. S7 for view of active-site residues in electron density), are similar to those seen in structures of other Usp enzymes bound to Ubal (21, 28, 29). Ubiquitin binds to the fingers domain of Ubp8, with its hydrophobic face (including I44) interacting with the β sheet, whereas the C-terminal tail of ubiquitin binds in a conserved groove that leads to the active site (Fig. 1, A and D). In the absence of ubiquitin, a number of structural differences occur in Ubp8. The fingers region that binds ubiquitin in the complex remains in an open conformation in apo DUBm (Fig. 4A), but the zinc-binding module and several β strand residues become less well ordered, which results in weak electron density that could not be completely traced (fig. S8A). Nonetheless, the fingers domain does not appear to collapse and occlude the ubiquitin-binding pocket, as is seen in the structure of the human USP8 apoenzyme (a Usp family member that is not an ortholog of yeast Ubp8) (30) (Fig. 4A). Most striking, however, are the changes that occur near the enzyme active site (Fig. 4B; electron

density shown in fig. S8). Seven Ubp8 residues located adjacent to the ubiquitin C terminus, 228 to 233, are disordered in the absence of ubiquitin binding (Fig. 4B). In addition, loop residues 421 to 426 move inward by up to 2.0 Å toward the ubiquitin tail-binding groove (Fig. 4B). The loop and the disordered region contain residues that contact ubiquitin directly; this suggests that binding of the ubiquitin C-terminal tail triggers the observed conformational change. Despite these conformational differences, the active-site residues (C146, H427, N443, and D444) are in their catalytically competent orientation in both the presence and absence of ubiquitin (Fig. 4B and fig. S8B). This is in contrast to deubiquitinating enzymes, such as HAUSP/USP7 (21), in which the active-site residues are not in a catalytically competent arrangement in the apoenzyme.

How does complex assembly activate Ubp8? In the absence of the other DUBm proteins, we find that Ubp8 is only about 1/200,000th as active as the intact DUBm (fig. S9) and shows substantial aggregation (fig. S10). A comparison of the DUBm structures with and without Ubal suggests that interactions among the DUBm proteins may stabilize a conformation of Ubp8 that is catalytically competent and able to bind ubiquitin. In the active-site region, the Sgf11 zinc finger directly contacts the loop in Ubp8 that contains the active-site cysteine, C146 (Fig. 4B). These contacts with Sgf11 may help to maintain the active-site residues in a catalytically competent conformation even when the neighboring residues (229 to 235) become disordered. Another way in which the other DUBm proteins may activate Ubp8 is by stabilizing a conformation of the fingers domain that favors binding of the globular portion of ubiquitin. The human USP8 apoenzyme (not an ortholog of yeast Ubp8), for example, has a partially occluded ubiquitin-

binding pocket that would require a conformational change to accommodate ubiquitin (Fig. 4A). Because the β sheet of the Ubp8 fingers region is in an open conformation even in the absence of ubiquitin (Fig. 4A), it is possible that interactions between Ubp8 and the other DUBm subunits help to maintain a conformation that favors ubiquitin binding. Interactions of the upper portion of the fingers domain with Sgf73 helix $\alpha 2$ and with Sus1 (Figs. 1A and 4C) form extensive contacts that may favor the open conformation of Ubp8. Sgf73 may also play a general role in stabilizing the conformation of the Usp domain. Ubp8 forms the most extensive interface with Sgf73, with a total area of 3075 Å², as compared with the other pairwise domain interactions in the DUBm. Note that Sgf73 is the “mortar” that holds together the two lobes of the DUBm complex (Fig. 4C); it promotes interactions between the two lobes and aligns Sgf11 and Sus1, which also contact the Usp domain at the interface between the two lobes. Although the structure of the Ubp8 Usp domain on its own is not known, it is possible that these extensive interactions with Sgf73 may also help to stabilize the overall USP fold or to affect protein dynamics in a way that favors the catalytically competent structure.

The structure of the SAGA DUBm suggests how this module interacts with its natural, *in vivo* substrate, monoubiquitinated histone H2B within a nucleosome. The electrostatic surface potential of the DUBm (Fig. 4D) reveals a basic region that could favor interactions with the negatively charged DNA when the nucleosome is positioned with ubiquitinated K123 of H2B in the active site of Ubp8. This region of the DUBm is positively charged because of the zinc finger module of Sgf11, which contains four basic residues (R78, R84, R91, and R95) (fig. S11) that are conserved in ATXN7L3, the human homolog of Sgf11. Two additional C-terminal arginine residues, R98 and R99, which are disordered in

the structure but are also conserved in the human homolog, would further contribute to the strong positive charge in this region. Figure S12 shows a model for how a yeast nucleosome (3I) monoubiquitinated at K123 of H2B can dock on the DUBm, with ubiquitin in the active site as seen in the Ubal-bound structure (Fig. 1D). This arrangement brings the basic patch on the DUBm in close approach with the DNA, which favors interactions with the sugar-phosphate backbone. The interdependent structural and functional roles of the four SAGA DUBm proteins in mediating biochemical activity, substrate binding, and incorporation into the larger SAGA complex is likely an example of how other subcomplexes of SAGA, and other coactivator and corepressor complexes, cooperate to regulate transcription.

References and Notes

1. E. I. Campos, D. Reinberg, *Annu. Rev. Genet.* **43**, 559 (2009).
2. E. Koutelou, C. L. Hirsch, S. Y. Dent, *Curr. Opin. Cell Biol.* (2010).
3. S. Rodríguez-Navarro, *EMBO Rep.* **10**, 843 (2009).
4. J. A. Daniel, P. A. Grant, *Mutat. Res.* **618**, 135 (2007).
5. R. Luthra *et al.*, *J. Biol. Chem.* **282**, 3042 (2007).
6. P. Pascual-García *et al.*, *Genes Dev.* **22**, 2811 (2008).
7. K. W. Henry *et al.*, *Genes Dev.* **17**, 2648 (2003).
8. A. Köhler, M. Schneider, G. G. Cabal, U. Nehrbass, E. Hurt, *Nat. Cell Biol.* **10**, 707 (2008).
9. K. K. Lee, S. K. Swanson, L. Florens, M. P. Washburn, J. L. Workman, *Epigenetics Chromatin* **2**, 2 (2009).
10. A. Köhler *et al.*, *Mol. Biol. Cell* **17**, 4228 (2006).
11. Y. Ye, H. Scheel, K. Hofmann, D. Komander, *Mol. Biosyst.* **5**, 1797 (2009).
12. K. K. Lee, L. Florens, S. K. Swanson, M. P. Washburn, J. L. Workman, *Mol. Cell. Biol.* **25**, 1173 (2005).
13. V. M. Weake *et al.*, *EMBO J.* **27**, 394 (2008).
14. B. S. Atanassov *et al.*, *Mol. Cell* **35**, 352 (2009).
15. H. J. Lee *et al.*, *Gene Expr. Patterns* **6**, 277 (2006).
16. K. Lindblad *et al.*, *Genome Res.* **6**, 965 (1996).
17. M. Latouche *et al.*, *Mol. Cell. Neurosci.* **31**, 438 (2006).
18. C. M. Pickart, I. A. Rose, *J. Biol. Chem.* **261**, 10210 (1986).
19. Materials and methods are available as supporting material on Science Online.
20. M. Hu *et al.*, *Cell* **111**, 1041 (2002).
21. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
22. D. Helmlinger, L. Tora, D. Devys, *Trends Genet.* **22**, 562 (2006).
23. F. E. Reyes-Turcu *et al.*, *Cell* **124**, 1197 (2006).
24. J. Bonnet, C. Romier, L. Tora, D. Devys, *Trends Biochem. Sci.* **33**, 369 (2008).
25. M. D. Allen, M. Bycroft, *Protein Sci.* **16**, 2072 (2007).
26. A. M. Ellisdon, D. Jani, A. Köhler, E. Hurt, M. Stewart, *J. Biol. Chem.* **285**, 3850 (2010).
27. D. Jani *et al.*, *Mol. Cell* **33**, 727 (2009).
28. M. Renatus *et al.*, *Structure* **14**, 1293 (2006).
29. M. Hu *et al.*, *EMBO J.* **24**, 3747 (2005).
30. G. V. Avvakumov *et al.*, *J. Biol. Chem.* **281**, 38061 (2006).
31. C. L. White, R. K. Suto, K. Luger, *EMBO J.* **20**, 5207 (2001).
32. We thank A. DiBello for helping with the ubiquitin aldehyde synthesis, E. Hurt for providing the Sus1 clone, S. Corcoran at the General Medicine and Cancer Institutes Collaborative Access Team (GM/CA-CAT) beamline at the Advanced Photon Source for assistance with data collection and processing, and M. Bianchet for advice and discussions. C.E.B. is supported by a Ruth Kirchstein Fellowship from the National Institute of General Medical Science, NIH (F32GM089037), and T.Y. is a Special Fellow of the Leukemia and Lymphoma Society. GM/CA-CAT has been funded in whole or in part with federal funds from the National Cancer Institute (Y1-CO-1020) and the National Institute of General Medical Science (Y1-GM-1104), NIH. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under contract no. DE-AC02-06CH11357. Coordinates and structure factors have been deposited in the Protein Data Bank with accession codes 3MHS (DUBm bound to Ubal) and 3MHH (apo DUBm).

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1190049/DC1
Materials and Methods
Figs. S1 to S12
Table S1
References

25 March 2010; accepted 8 April 2010
Published online 15 April 2010;
10.1126/science.1190049
Include this information when citing this paper.

Network Diversity and Economic Development

Nathan Eagle,^{1,2*} Michael Macy,^{3,4} Rob Claxton^{1,5}

Social networks form the backbone of social and economic life. Until recently, however, data have not been available to study the social impact of a national network structure. To that end, we combined the most complete record of a national communication network with national census data on the socioeconomic well-being of communities. These data make possible a population-level investigation of the relation between the structure of social networks and access to socioeconomic opportunity. We find that the diversity of individuals' relationships is strongly correlated with the economic development of communities.

Theoretical work suggests that the structure of social relations between individuals may affect a community's economic development. More precisely, economic opportunities are

more likely to come from contacts outside a tightly knit local friendship group. Hence, highly clustered, or insular, social ties are predicted to limit access to social and economic prospects

from outside the social group, whereas heterogeneous social ties may generate these opportunities from a range of diverse contacts (1, 2). To date, however, the correspondence between network diversity and a population's economic well-being has not been quantified, largely because of the inability to obtain data that includes measures of network structure and economic development at the population level (3, 4). These data limitations have constrained related research to quantifying the effects of individual network-

¹Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501, USA. ²MIT Media Laboratory, Massachusetts Institute of Technology, 20 Ames Street, Cambridge, MA 02139, USA. ³Department of Sociology, Cornell University, Ithaca, NY 14853, USA. ⁴The Brookings Institution, 1775 Massachusetts Avenue, NW, Washington, DC 20036, USA. ⁵BT Group plc, Ipswich IP5 3RE, UK.

*To whom correspondence should be addressed. E-mail: nathan@mit.edu

tie formation. Previous studies have found that individuals benefit from having social ties that bridge between communities. These benefits include access to jobs and promotions (5–13), greater job mobility (14, 15), higher salaries (9, 16, 17), opportunities for entrepreneurship (18, 19), and increased power in negotiations (20, 21). Although these studies suggest the possibility that the individual-level benefits of having a diverse social network may scale to the population level, the relation between network structure and community economic development has never been directly tested (22).

As policy-makers struggle to revive ailing economies, understanding this relation between network structure and economic development may provide insights into social alternatives to traditional stimulus policies. To that end, we analyzed the most complete record of a national communication network studied to date and coupled this social network data with detailed socioeconomic indicators to measure this relation directly, at the population level. The communication network data were collected during the month of August 2005 in the UK. The data contain more than 90% of the mobile phones and greater than 99% of the residential and business landlines in the country. The resulting network has 65×10^6 nodes, 368×10^6 reciprocated social ties, a mean geodesic distance (minimum number of direct or indirect edges connecting two nodes) of 9.4, an average degree of 10.1 network neighbors, and a giant component (the largest connected subgraph) containing 99.5% of all nodes (23).

Although the nature of this communication data limits causal inference, we were able to test the hypothesized correspondence between social network structure and economic development using the 2004 UK government's Index of Multiple Deprivation (IMD), a composite measure of relative prosperity of 32,482 communities encompassing the entire country (24), based on income, employment, education, health, crime, housing, and the environmental quality of each region (25). Each residential landline number was associated with the IMD rank of the exchange in which it was located, as shown in Fig. 1. Obtaining the socioeconomic profile for a given telephone exchange area involves aggregating over the census regions within the exchange area. First we uniquely mapped each census region to the exchange area with which it has the greatest spatial overlap. We subsequently aggregated, for each exchange area, the population-weighted average of the IMD for the census regions assigned to each exchange:

$$\mu_{\text{weighted}} = \sum_{i=1}^n w_i x_i \quad (1)$$

and

$$\sigma_{\text{weighted}}^2 = \sum_{i=1}^n w_i (x_i - \mu_{\text{weighted}})^2 \quad (2)$$

where x_i is the census rank for the i th census region that makes up the exchange area and w_i is the population weight of the i th census region given by the fraction of the total population of the exchange area residing in the i th census region.

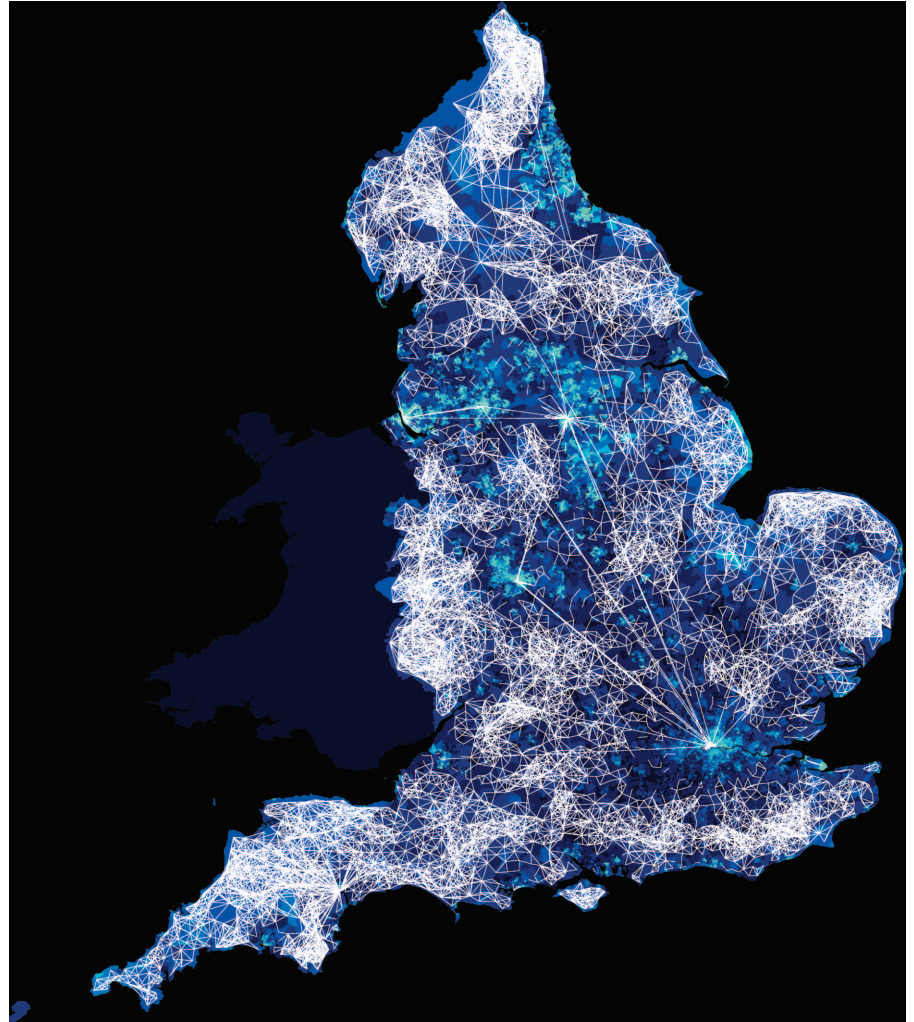


Fig. 1. An image of regional communication diversity and socioeconomic ranking for the UK. We find that communities with diverse communication patterns tend to rank higher (represented from light blue to dark blue) than the regions with more insular communication. This result implies that communication diversity is a key indicator of an economically healthy community. [(29) Crown copyright material is reproduced with the permission of the Controller of Her Majesty's Stationery Office]

We then compared the IMD rank of each community with diversity metrics associated with each member's social network. Mobile numbers were included (along with landlines) in the calculation of the nonspatial diversity measures; however, they were not used to identify members of a community (because of insufficient data on spatial location). We developed two new metrics to capture the social and spatial diversity of communication ties within an individual's social network. We quantify topological diversity as a function of the Shannon entropy,

$$H(i) = - \sum_{j=1}^k p_{ij} \log(p_{ij}) \quad (3)$$

where k is the number of i 's contacts and p_{ij} is the proportion of i 's total call volume that involves j , or

$$p_{ij} = \frac{V_{ij}}{\sum_{j=1}^k V_{ij}} \quad (4)$$

where V_{ij} is the volume between node i and j . We then define social diversity, $D_{\text{social}}(i)$, as the Shannon

entropy associated with individual i 's communication behavior, normalized by k :

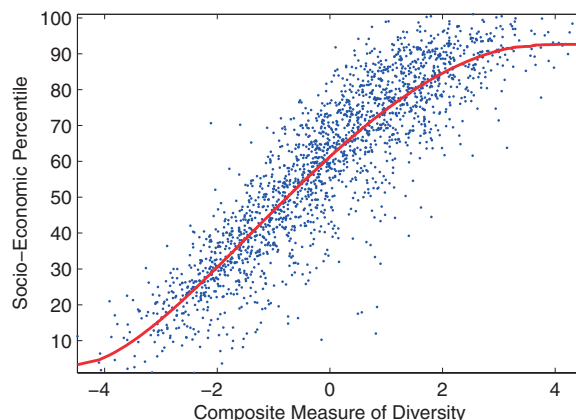
$$D_{\text{social}}(i) = \frac{- \sum_{j=1}^k p_{ij} \log(p_{ij})}{\log(k)} \quad (5)$$

The above measure of topological diversity does not take into account the geographic diversity in the calling patterns within a community. We define a similar measure for spatial diversity, $D_{\text{spatial}}(i)$, by replacing call volume with the geographic distance spanned by an individual's ties to the 1992 telephone exchange areas in the UK,

$$D_{\text{spatial}}(i) = \frac{- \sum_{a=1}^A p_{ia} \log(p_{ia})}{\log(A)} \quad (6)$$

in which p_{ia} is the proportion of time i spends communicating with a th of A total exchange areas. High diversity scores imply that an individual splits her time more evenly among social ties and between different regions.

Fig. 2. The relation between social network diversity and socioeconomic rank. Diversity was constructed as a composite of Shannon entropy and Burt's measure of structural holes, by using principal component analysis. A fractional polynomial was fit to the data.



Although both social and spatial network diversity scores were strongly correlated with IMD rank ($r = 0.73$ and $r = 0.58$, respectively), we found a weaker positive correlation present using number of contacts ($r = 0.44$) and a negative correlation for communication volume ($r = -0.33$). For example, whereas inhabitants of Stoke-on-Trent, one of the least prosperous regions in the UK, averaged a higher monthly call volume than the national average, they have one of the lowest diversity scores in the country. Similarly prosperous Stratford-upon-Avon has inhabitants with extremely diverse networks, despite no more communication than the national average.

The strong association between diversity and IMD rank persists using other network diversity metrics, including Burt's measure of "structural holes" (9). A structural hole is a missing relation between any two of a node's neighbors, creating an open triad. Burt's seminal work showed that remuneration within an organization increases with the number of structural holes that surround a node. Our results show that this relation scales to the level of communities, whose socioeconomic opportunities increase with the number of structural holes in the ego networks of the members ($r = 0.72$). Moreover, a composite measure constructed via principal component analysis was an even better predictor of economic development than either component alone, as illustrated in Fig. 2 ($r = 0.78$).

By coupling the most complete population-level social network studied to date with community-level economic outcomes, we were able to validate a central assumption that is widely accepted in network science but was untested at the population level: Do more diverse ties provide greater access to social and economic opportunities? Although the causal direction of this relation—whether network diversity promotes opportunity or economic development leads to more diversified contacts—cannot be established, social network diversity seems to be at the very least a strong structural signature for the economic development of a community. On a population level, the surprisingly strong correspondence we discovered between the structure of social contacts and the economic well-being of populations highlights the potential benefit of socially targeted policies for economic development. However, additional research will be required to derive reliable

policy implications. In particular, establishing the causal mechanisms underlying the observed correspondence between network diversity and economic development may require additional longitudinal social network and economic data (26–28).

References and Notes

1. M. Newman, *SIAM Rev.* **45**, 167 (2003).
2. S. Page, *The Difference: How the Power of Diversity Creates Better Groups, Firms, Schools, and Societies* (Princeton Univ. Press, Princeton, NJ, 2007).
3. M. Granovetter, *Sociol. Theory* **1**, 201 (1983).
4. Y. Bian, *Am. Sociol. Rev.* **62**, 366 (1997).
5. M. Granovetter, *Am. J. Sociol.* **78**, 1360 (1973).
6. R. Fernandez, H. Weinberg, *Stanford GSB Research Paper Series no. 1382*, 1 (1994).
7. D. Brass, *Adm. Sci. Q.* **29**, 518 (1984).
8. D. Brass, *Acad. Manage. J.* **28**, 327 (1985).
9. R. Burt, *Structural Holes: The Social Structure of Competition* (Harvard Univ. Press, Cambridge, MA, 1992).
10. P. Marsden, J. Hurlbert, *Soc. Forces* **66**, 1038 (1988).
11. N. Lin, W. Ensel, J. Vaughn, *Am. Sociol. Rev.* **46**, 393 (1981).
12. H. Flap, N. Degraaf, *Netherlands J. Sociol.* **22**, 145 (1986).
13. N. Degraaf, H. Flap, *Soc. Forces* **67**, 453 (1988).
14. B. Wegener, *Am. Sociol. Rev.* **56**, 60 (1991).
15. J. Podolny, J. Baron, *Am. Sociol. Rev.* **62**, 673 (1997).
16. M.-D. Seidel, J. Polzer, K. Stewart, *Adm. Sci. Q.* **45**, 1 (2000).
17. S. Seibert, M. Kraimer, R. Liden, *Acad. Manage. J.* **44**, 219 (2001).
18. H. Aldrich, C. Zimmer, in *The Art and Science of Entrepreneurship*, D. L. Sexton, R. W. Smilor, Eds. (Ballinger Publishing, Cambridge, MA, 1986), pp. 3–24.
19. P. Dubini, H. Aldrich, *J. Bus. Venturing* **6**, 305 (1991).
20. M. Burkhardt, D. Brass, *Adm. Sci. Q.* **35**, 104 (1990).
21. D. Brass, M. Burkhardt, *Acad. Manage. J.* **36**, 441 (1993).
22. Previous studies have used longitudinal designs to test the hypothesized one-way causal direction between network position and individual economic benefits. These results suggest a similar causal direction at the population level, but our national data do not allow us to test for causal direction, and we cannot rule out the possibility that economic advantages may also lead to changes in network structure.
23. The anonymized call logs were recorded by the network operator as required by law and for billing purposes and not for the purpose of this project. Although these communication data and the exact location of the telephone exchanges are not publicly available, the regional aggregates are available from the first author.
24. Department of Communities and Local Government, *Indices of Deprivation 2004—Summary (revised)* (Department of Communities and Local Government, Stationery Office, London, 2004); www.communities.gov.uk/archived/general-content/communities/indicesofdeprivation/indicesofdeprivation?view=Standard.
25. The 2004 IMD was constructed before the 2005 telephone call logs were recorded. We assume that the 2004 IMD rankings are relatively constant and, therefore, make no causal inference from the temporal order. Additionally, exchange areas can also be scored with specific IMD components, such as education. Higher education may promote more diverse network ties and lead to jobs with higher income, which might account for part of the correspondence between IMD and network diversity.
26. S. Aral, L. Muchnik, A. Sundararajan, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21544 (2009).
27. S. Currarini, M. O. Jackson, P. Pin, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 4857 (2010).
28. C. Manski, *Rev. Econ. Stud.* **60**, 531 (1993).
29. Office for National Statistics, *Super Output Area Boundaries* (Her Majesty's Stationery Office, London, 2004).
30. This work was supported by the Santa Fe Institute, the U.S. NSF (BCS-0537606), and British Telecom.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5981/1029/DC1
Materials and Methods

Figs. S1 to S6

References

4 January 2010; accepted 14 April 2010

10.1126/science.1186605

Coadministration of a Tumor-Penetrating Peptide Enhances the Efficacy of Cancer Drugs

Kazuki N. Sugahara,^{1*} Tabet Teesalu,^{1*} Priya Prakash Karmali,² Venkata Ramana Kotamraju,¹ Lilach Agemy,¹ Daniel R. Greenwald,³ Erkki Ruoslahti^{1,2†}

Poor penetration of anticancer drugs into tumors can be an important factor limiting their efficacy. We studied mouse tumor models to show that a previously characterized tumor-penetrating peptide, iRGD, increased vascular and tissue permeability in a tumor-specific and neuropilin-1–dependent manner, allowing coadministered drugs to penetrate into extravascular tumor tissue. Importantly, this effect did not require the drugs to be chemically conjugated to the peptide. Systemic injection with iRGD improved the therapeutic index of drugs of various compositions, including a small molecule (doxorubicin), nanoparticles (nab-paclitaxel and doxorubicin liposomes), and a monoclonal antibody (trastuzumab). Thus, coadministration of iRGD may be a valuable way to enhance the efficacy of anticancer drugs while reducing their side effects, a primary goal of cancer therapy research.

The therapeutic efficacy of many anticancer drugs is limited by their poor penetration into tumor tissue and by their adverse ef-

fects on healthy cells, which limits the dose of drug that can be safely administered to cancer patients. In solid tumors, many anticancer drugs

penetrate only 3 to 5 cell diameters from the blood vessels, leading to reduced efficacy and the development of drug resistance (1, 2). We recently identified a tumor-penetrating peptide, iRGD (CRGDK/RGPD/EC) (3), that, when chemically conjugated to a drug, can carry the drug deep into extravascular tumor tissue (4). Like conventional RGD peptides, iRGD homes to tumors by initially binding to α_v integrins that are specifically expressed on the endothelium of tumor vessels (4–6). iRGD is then proteolytically cleaved in the tumor to produce CRGDK/R. The truncated peptide loses much of its integrin-binding activity, but gains affinity for neuropilin-1 (NRP-1) because of the C-terminal exposure of a conditional C-end Rule (CendR) motif (R/KXXR/K) (7). The NRP-1 binding triggers tissue penetration, which

is tumor-specific because the cleavage requires earlier binding of the peptide to integrins. These features confer on iRGD a tumor-specific tissue penetration activity. Here we explore whether the iRGD peptide can enhance cancer drug delivery and activity when it is administered as a combination therapy with drugs that are not chemically conjugated to it. This would be advantageous because already approved drugs could be used without creating a new chemical entity, and because coupling often interferes with drug activity.

The activity of peptides and proteins that bind to NRP-1 through a C-terminal CendR motif can be demonstrated by monitoring vascular permeability (7–9). We examined whether the CendR-triggered vascular permeability may play a role in the iRGD tumor-penetrating activities using human tumor xenografts growing in immunodeficient mice and in de novo mouse tumors growing in transgenic mice. Indeed, chemically synthesized iRGD peptide, when co-injected with the albumin-binding dye Evans blue (10–12), caused tumor-specific accumulation of the dye in all five tumor models that we tested (one breast cancer, two prostate cancers, and two pancreatic adenocarcinomas), including secondary invasion sites and disseminated tumors (figs. S1A and S2, A and B). The accumulation of the dye was dependent on the dose of iRGD administered and peaked at ~400%

more than the dye alone (fig. S1B). Non-CendR RGD peptides, RGD-4C (CDCRGDCFC) (13) and cyclo(-RGDfK-) (14), and a scrambled iRGD with no CendR motif (CRGDDGPKC) (here, bold letters indicate the position swap of amino acids) did not increase the permeability (figs. S1C and S2C). Preinjection of an antibody to NRP-1 (anti-NRP-1) inhibited the iRGD-induced permeability (fig. S1D). CRGDK, the truncated iRGD peptide with an exposed CendR motif, enhanced local vascular permeability in the skin in a dose-dependent manner (fig. S3) (7). When injected systemically, CRGDK increased permeability in the tumors, as well as in the lungs and heart. CRGDK was somewhat selective for the tumors, probably because of its residual affinity to integrins and the generally high expression of NRP-1 in tumors (fig. S1C) (4, 7, 15).

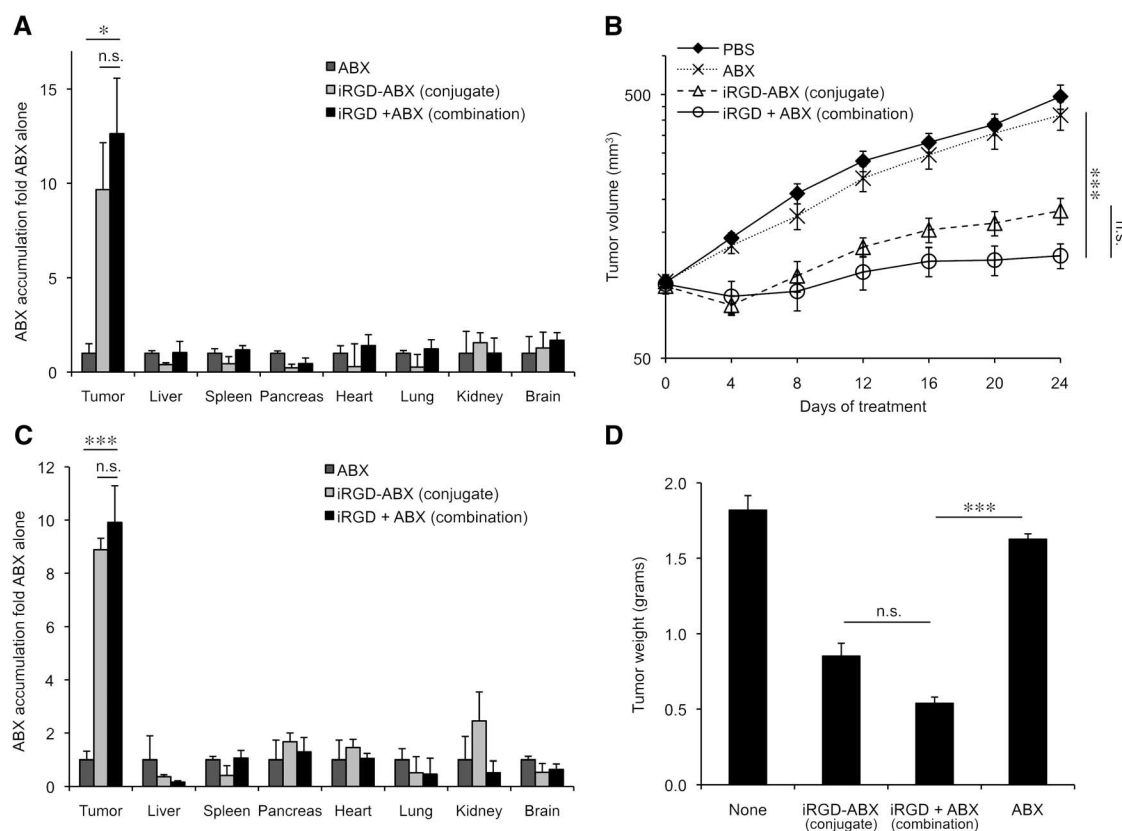
The tumor-specific increase in tissue access mediated by iRGD suggests a way of improving the delivery of compounds to tumor parenchyma (fig. S4). A fluorescein-labeled non-CendR peptide CRGDC (FAM-CRGDC; 1.3 kD), which minimally penetrates tumors by itself (4, 16), showed enhanced extravascular distribution upon iRGD co-injection (fig. S5A). The co-injection caused a 300% increase in both of the FAM-CRGDC-positive areas (fig. S5B) and the spreading of FAM-CRGDC (fig. S5C) in the tumors. We ob-

¹Vascular Mapping Laboratory, Center for Nanomedicine, Sanford-Burnham Medical Research Institute at University of California at Santa Barbara, Biology II Building, University of California, Santa Barbara, CA 93106–9610, USA. ²Cancer Research Center, Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA. ³Santa Barbara Hematology Oncology Medical Group, Cancer Center of Santa Barbara, 540 West Pueblo Street, Santa Barbara, CA 93105, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: ruoslahti@burnham.org

Fig. 1. Comparison of the drug-delivery efficiency of the iRGD combination regimen and conjugated drug delivery. (A and C) Nab-paclitaxel (ABX) quantification in orthotopic human breast tumor (BT474) (A) and human prostate tumor (22Rv1) (C) xenograft models. ABX with free iRGD (combination), ABX coated with iRGD (conjugate), or ABX alone, was intravenously injected into tumor-bearing mice. Three hours later, ABX was captured from tumor extracts with an antitaxol antibody, followed by detection with a human albumin antibody. $n = 3$ mice per group for both (A) and (C). (B and D) Long-term treatment of tumor mice with ABX. Mice bearing orthotopic BT474 (B) or 22Rv1 (D) tumors were intravenously injected with the indicated ABX formulations every other day at 3 mg paclitaxel/kg per injection or with phosphate-buffered saline (PBS) only. The treatment was continued for both 24 (B) and 16 days (D). $n = 8$ per group (B) and 9 per group (D), respectively. Statistical analyses were



performed with Student's *t* test in (A) and (C) and analysis of variance (ANOVA) in (B) and (D). Error bars denote mean $\pm$ SEM. n.s., not significant; * $P < 0.05$; *** $P < 0.001$.

tained similar results with 3- and 10-kD dextrans (fig. S5). Two nanoparticles, iron oxide nanoworms (17) and T7 phage, also extravasated and showed enhanced accumulation in the tumor upon iRGD co-injection (fig. S6). These results show that iRGD can increase the tumor accumulation of compounds with different sizes and chemical properties.

Intravenously injected molecules and nanoparticles slowly extravasate into perivascular areas in tumor tissue through passive leakage (18, 19). The rapid and deep tumor penetration of the probes co-injected with iRGD prompted us to study the possibility that CendR might be triggering an active transport process. To exclude circulatory effects, we used fresh explants of PPC-1 human prostate tumors in this study. Phage particles expressing the iRGD peptide on their surface (4), but not inert control phage, penetrated into PPC-1 prostate cancer xenograft explants, advancing ~4 mm in 90 min (fig. S7). The penetration was inhibited by anti-NRP-1, sodium azide, and by lowering the temperature, suggesting that the penetration process was NRP-1- and energy-dependent. Importantly, inert phage penetrated into the explants in the presence of iRGD, but not with a control peptide.

To study the effect of iRGD on drug delivery and efficacy, we administered the peptide as a combination therapy with several types of cancer drugs to mice bearing human tumor xenografts. We first compared the delivery efficiency of this combination regimen to that of the cancer drug

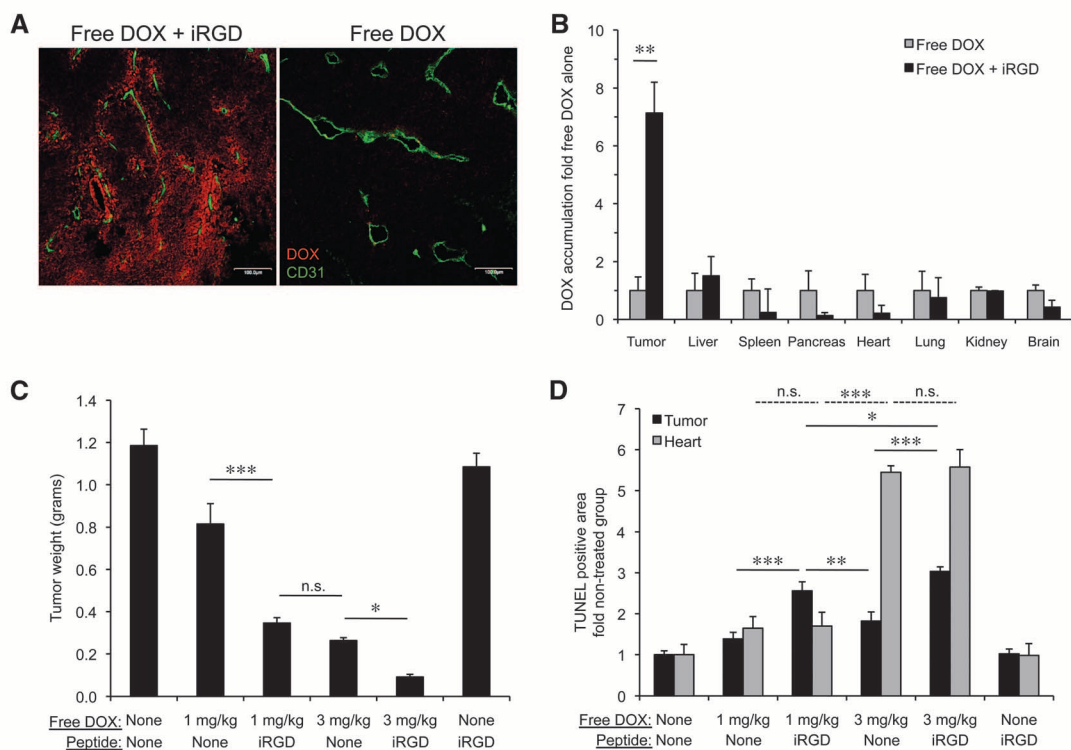
alone and to that of the cancer drug when it was chemically conjugated to the peptide. When intravenously injected with iRGD, Nab-paclitaxel [abraxane (ABX)], a 130-nm nanoparticle consisting of albumin-embedded paclitaxel (4, 20), accumulated 12-fold more in orthotopic BT474 human breast tumors than ABX given alone and penetrated far from the tumor vessels (Fig. 1A and fig. S8). Treatment of mice every other day with the ABX/iRGD combination inhibited the growth of BT474 tumors, which were resistant to equivalent doses of ABX alone (Fig. 1B). The combination was slightly more effective than the conjugated drug in inhibiting tumor growth, as well as in tumor accumulation, but the differences did not reach statistical significance. Similar results were obtained in orthotopic 22Rv1 human prostate tumors (Fig. 1, C and D).

We next used another clinically relevant system to evaluate the effect of the combination therapy on the therapeutic index (a comparison of the dose needed to elicit a therapeutic effect to the lethal dose). Doxorubicin (DOX) and DOX liposomes have clinical activity in a number of tumor types, including prostate cancer (21). We treated orthotopic 22Rv1 tumors with a combination of iRGD and DOX (0.6 kD). Free DOX co-injected with iRGD penetrated the tumors and accumulated sevenfold more in the tumors than DOX given alone (Fig. 2, A and B). The combination therapy that included 1 mg/kg DOX was as potent as 3 mg/kg DOX alone, a dose that reaches the cumulative maximum tolerated dose (Fig. 3C)

(22). Combining iRGD with 3 mg/kg of DOX potentiated the activity of DOX, inducing nearly complete tumor-growth inhibition (Fig. 2C). Tumors from both the 1- and 3-mg/kg DOX groups combined with iRGD showed stronger terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining, an indicator of cell death, than tumors treated with 3-mg/kg DOX alone (Fig. 2D). Cardiotoxicity, the main dose-limiting toxicity of DOX, is manifested as cardiomyocyte apoptosis (23). The iRGD combination had the same cardiotoxicity as an equivalent dose of DOX alone (Fig. 2D). The body-weight shift was also the same, whether DOX was given alone or combined with iRGD (fig. S9). Similar results were obtained with orthotopic 4T1 mouse breast tumors (figs. S9 and S10). iRGD alone at the dose used in the combination group showed no effect on tumor growth (Fig. 2C), supporting the notion that the effects of the combination are due to improved drug penetration.

We also tested DOX in a liposomal formulation (particle diameter = 120 nm). The results were similar to those with free DOX with regard to treatment efficacy (Fig. 3A), tumor accumulation of the drug (14-fold) (Fig. 3B), tumor penetration (fig. S11), and toxicity (Fig. 3C and fig. S9). In addition, we tested a non-CendR RGD in this system and found that it did not enhance the efficacy of the drug (Fig. 3A). These results indicate that, in comparison to drug administered alone, the iRGD combination therapy provides

Fig. 2. Enhanced antitumor effect of free DOX co-injected with iRGD. (A and B) Mice bearing orthotopic 22Rv1 human prostate tumors were intravenously injected with a mixture of DOX (10 mg/kg) and 4 μ mol/kg of iRGD or PBS. Tumors and tissues were collected 1 hour later. *n* = 3 per group. In (A), the tumors were sectioned and stained for blood vessels with anti-CD31, and the native fluorescence was used to detect DOX. Scale bars, 100 μ m. In (B), DOX in the tissues was quantified. (C) Mice bearing orthotopic 22Rv1 tumors implanted 2 weeks earlier received intravenous injections of DOX (1 or 3 mg/kg) or PBS, combined with 4 μ mol/kg of iRGD or PBS, every other day. The tumors were harvested and weighed after 24 days of treatment. *n* = 10 per group. (D) TUNEL staining was performed on tumors and hearts from the treatment study and was quantified for positive staining. Statistical analyses were performed with Student's *t* test (B) and ANOVA (C and D). Error bars denote mean $\pm$ SEM. n.s., not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.



equivalent or better antitumor efficacy at a three-fold lower dose of the drug, with a commensurate reduction in toxicity. The results also show that this therapy regimen can be effective with drugs ranging in size from a small-molecular weight chemical (DOX) to nanoparticles (ABX, DOX liposomes).

Finally, we tested the iRGD-combination regimen in mice bearing tumors derived from BT474 human breast tumor cells, which over-express HER2/neu/ErbB2. This growth-factor receptor is the target of trastuzumab, a therapeutic monoclonal antibody in clinical use for the treatment of breast cancer (24, 25). Co-injection of iRGD resulted in a 14-fold increase in trastuzumab-positive areas within the tumor (Fig. 4A). An enzyme-linked immunosorbent assay (ELISA) showed that iRGD enhanced the accumulation of trastuzumab in the tumors 40-fold; this increase is probably due to enhanced access and binding of the antibody to the tumor cells (Fig. 4B). Combining trastuzumab with iRGD increased the drug potency at different trastuzumab dose levels (Fig. 4C). At a clinical dose (9 mg/kg), the combination eradicated all tumors in the mice, whereas the equivalent dose of trastuzumab alone only slowed tumor growth (Fig. 4C). The eradicated tumors did not relapse during a 2-week observation period after the treatment was stopped.

The iRGD-mediated enhancement in drug penetration persisted even after more than 3 weeks of antibody treatment (figs. S12 and S13 for DOX and trastuzumab, respectively). However, the difference between the combination and the drug alone was smaller than with single dosing (compare fig. S13B with Fig. 4A). This explains the lack of a linear relation between the single dosing of the drugs, which gave 7- to 40-fold more drug accumulation, and the long-term treatments, which gave 3-fold stronger antitumor effects.

We also tested the possibility that iRGD might have the adverse effect of enhancing metastasis by promoting intravasation of tumor cells. No macroscopic metastases were found in mice treated with iRGD. Immunofluorescence for human leukocyte antigens and quantitative polymerase chain reaction measuring human genomic DNA revealed no human cells in organs collected at the end of the treatments, and the low levels of DNA were similar in the iRGD and control groups in the BT474 and 22Rv1 models (figs. S14 to S16) (26, 27).

The CendR penetration effect of the tumor-targeting methodology described here is distinct from the enhanced permeability and retention (EPR) effect: (i) EPR is a passive leakage from tumor vessels (18, 19), whereas the CendR penetration is receptor-mediated and energy-dependent. (ii) CendR is more effective than the EPR (fig. S5). (iii) CendR causes extravasation within minutes, whereas EPR requires 6 to 8 hours to peak (4, 18). (iv) Finally, CendR is effective with small molecules, whereas EPR is not (18). However, there may be a CendR component in EPR, be-

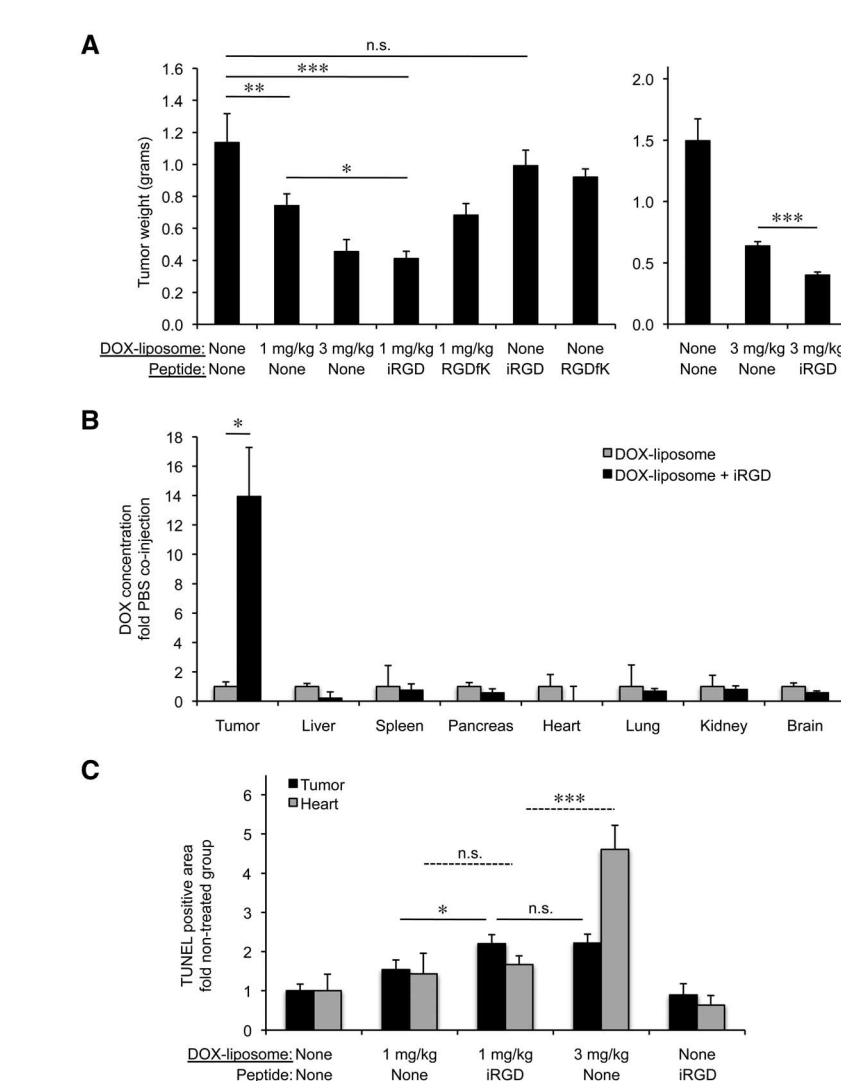


Fig. 3. Enhanced antitumor effect of DOX liposomes co-injected with iRGD. **(A)** Nude mice bearing orthotopic 22Rv1 human prostate tumors implanted 2 weeks earlier received daily intravenous injections of DOX liposomes (1 or 3 mg/kg) or PBS, combined with 2 μ mol/kg of iRGD or cyclo(-RGDfK-) or PBS. The tumors were harvested and weighed after 17 days of treatment. $n = 5$ per group (left panel); $n = 8$ per group (right panel). **(B)** Mice bearing orthotopic 22Rv1 tumors were intravenously injected with DOX liposomes (5 mg/kg), followed by 4 μ mol/kg of iRGD or PBS. The tumors and tissues were collected 3 hours later, and DOX in the tissues was quantified. $n = 3$ per group. **(C)** TUNEL staining was performed on tumors and hearts from the treatment study in (A) and was quantified for positive staining. Statistical analyses were performed with ANOVA [(A) left panel, and (C)] and Student's t test [(A) right panel, and (B)]. Error bars denote mean $\pm$ SEM. n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

cause vascular endothelial growth factor-165 (VEGF-165), which is involved in the process, has an active CendR motif (7, 8, 18).

Administration of the tumor-penetrating peptide as a combination therapy bypasses two limitations of strategies in which the peptide is conjugated to the drug and delivered as a single agent: (i) The combination therapy does not require drug modification, which may impair drug activity, and (ii) the capacity of the system is not exceeded as easily as that of the conjugate delivery. Whereas delivery of the conjugated drug

depends on a finite number of target receptors, delivery of the peptide and drug as separate entities triggers a bulk transfer of the bystander drugs into the tumor tissue (28).

Attempts have been made to permeabilize tumor vasculature with VEGF and bradykinin for enhanced access of blood-borne molecules to tumor interstitium (29, 30), but the tumor specificity has been limited. One possible advantage of iRGD is that systemic administration of this peptide results in a tissue penetration effect that appears to be selective for tumors.

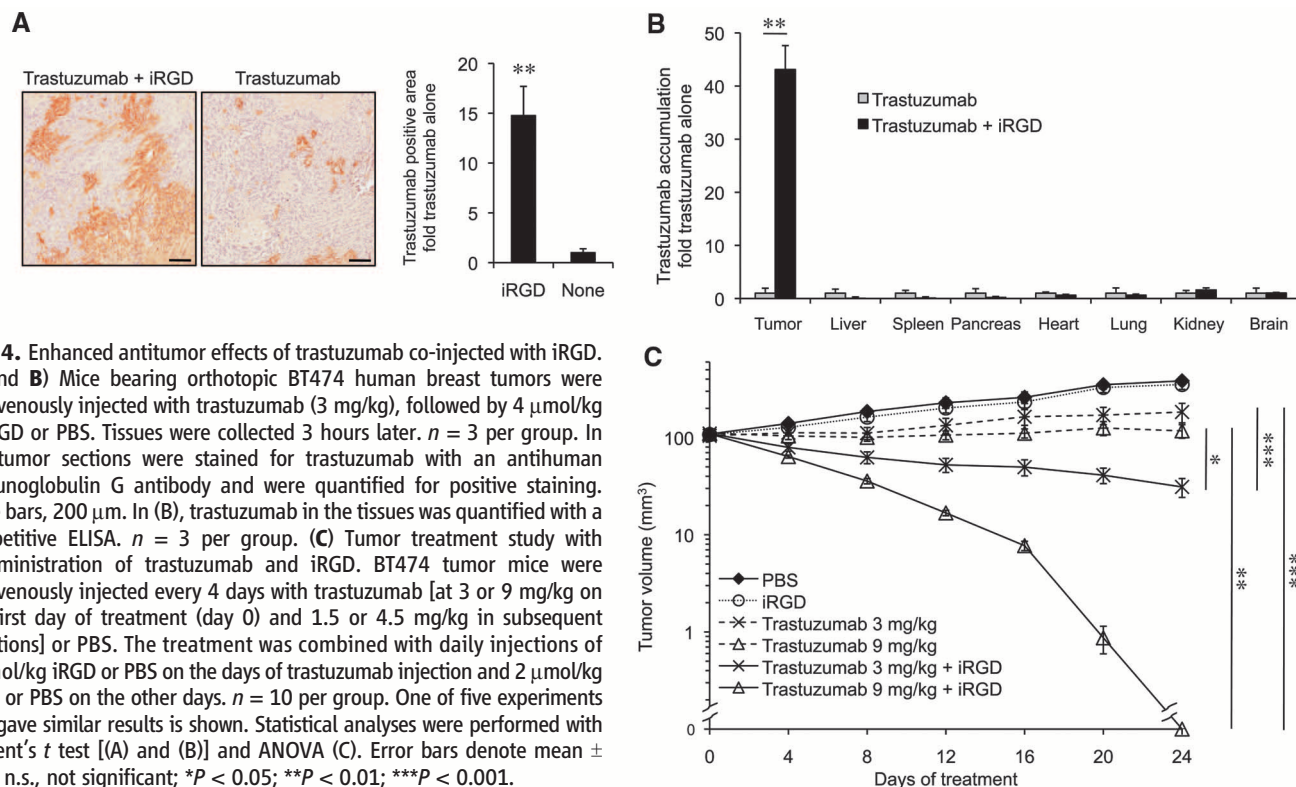


Fig. 4. Enhanced antitumor effects of trastuzumab co-injected with iRGD. **(A and B)** Mice bearing orthotopic BT474 human breast tumors were intravenously injected with trastuzumab (3 mg/kg), followed by 4 μ mol/kg of iRGD or PBS. Tissues were collected 3 hours later. $n = 3$ per group. In **(A)**, tumor sections were stained for trastuzumab with an antihuman immunoglobulin G antibody and were quantified for positive staining. Scale bars, 200 μ m. In **(B)**, trastuzumab in the tissues was quantified with a competitive ELISA. $n = 3$ per group. **(C)** Tumor treatment study with coadministration of trastuzumab and iRGD. BT474 tumor mice were intravenously injected every 4 days with trastuzumab [at 3 or 9 mg/kg on the first day of treatment (day 0) and 1.5 or 4.5 mg/kg in subsequent injections] or PBS. The treatment was combined with daily injections of 4 μ mol/kg iRGD or PBS on the days of trastuzumab injection and 2 μ mol/kg iRGD or PBS on the other days. $n = 10$ per group. One of five experiments that gave similar results is shown. Statistical analyses were performed with Student's t test **(A and B)** and ANOVA **(C)**. Error bars denote mean $\pm$ SEM. n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

We observed enhanced tumor-specific delivery of the 10 different compounds that we tested (from a 0.6-kD molecule up to a 130-nm particle), suggesting that the iRGD peptide might help improve the performance of a wide range of cancer drugs or tumor-imaging agents. Conceivably, the system can be further improved—for example, by employing multimeric iRGD, structurally stabilized iRGD, other tumor-homing CendR peptides (31–34) or peptide combinations, or by optimization of the dosing and administration schedules. Moreover, the efficacy and the specificity of the iRGD-mediated delivery remain to be shown in human patients. Human tumor vessels and tumor cells express the relevant α_v integrins (5, 6) and NRP-1 (15), and iRGD and its fragment bind to them (4), suggesting that the system will translate to the treatment of human cancer. Increasing the therapeutic index of standard therapy may enhance efficacy while reducing toxicity, the primary goal of modern cancer therapy.

References and Notes

1. T. W. Hambley, W. N. Hait, *Cancer Res.* **69**, 1259 (2009).
2. A. I. Minchinton, I. F. Tannock, *Nat. Rev. Cancer* **6**, 583 (2006).
3. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and X, any amino acid.
4. K. N. Sugahara *et al.*, *Cancer Cell* **16**, 510 (2009).
5. E. Ruoslahti, *Nat. Rev. Cancer* **2**, 83 (2002).
6. J. S. Desgrosellier, D. A. Cheresh, *Nat. Rev. Cancer* **10**, 9 (2010).
7. T. Teesalu, K. N. Sugahara, V. R. Kotamraju, E. Ruoslahti, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16157 (2009).
8. H. Jia *et al.*, *J. Biol. Chem.* **281**, 13493 (2006).
9. L. M. Acevedo, S. Barillas, S. M. Weis, J. R. Göthert, D. A. Cheresh, *Blood* **111**, 2674 (2008).
10. See supporting material on Science Online for methods and additional data. Statistical analyses are summarized in table S1.
11. A. A. Miles, E. M. Miles, *J. Physiol.* **118**, 228 (1952).
12. T. Murohara *et al.*, *Circulation* **97**, 99 (1998).
13. E. Koivunen, B. Wang, E. Ruoslahti, *Biotechnology (N.Y.)* **13**, 265 (1995).
14. E. A. Murphy *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 9343 (2008).
15. C. Pellet-Mary, P. Frankel, H. Jia, I. Zachary, *Biochem. J.* **411**, 211 (2008).
16. E. Koivunen, D. A. Gay, E. Ruoslahti, *J. Biol. Chem.* **268**, 20205 (1993).
17. J. H. Park *et al.*, *Small* **5**, 694 (2009).
18. H. Maeda, J. Fang, T. Inutsuka, Y. Kitamoto, *Int. Immunopharmacol.* **3**, 319 (2003).
19. F. Yuan *et al.*, *Cancer Res.* **55**, 3752 (1995).
20. P. P. Karmali *et al.*, *Nanomedicine* **5**, 73 (2009).
21. R. Petrioli, A. I. Fiaschi, E. Francini, A. Pascucci, G. Francini, *Cancer Treat. Rev.* **34**, 710 (2008).
22. F. Pastorino *et al.*, *Clin. Cancer Res.* **14**, 7320 (2008).
23. O. J. Arola *et al.*, *Cancer Res.* **60**, 1789 (2000).
24. B. M. Fendly *et al.*, *Cancer Res.* **50**, 1550 (1990).
25. P. Carter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4285 (1992).
26. T. Primeau *et al.*, *Immunol. Res.* **32**, 109 (2005).
27. D. A. Tagle, F. S. Collins, *Hum. Mol. Genet.* **1**, 121 (1992).
28. E. Ruoslahti, S. N. Bhatia, M. J. Sailor, *J. Cell Biol.* **188**, 759 (2010).
29. W. L. Monsky *et al.*, *Cancer Res.* **59**, 4129 (1999).
30. K. L. Black, N. S. Ningaraj, *Cancer Control* **11**, 165 (2004).
31. J. A. Hoffman *et al.*, *Cancer Cell* **4**, 383 (2003).
32. J. A. Joyce *et al.*, *Cancer Cell* **4**, 393 (2003).
33. P. Laakkonen, K. Porkka, J. A. Hoffman, E. Ruoslahti, *Nat. Med.* **8**, 751 (2002).
34. K. Porkka, P. Laakkonen, J. A. Hoffman, M. Bernasconi, E. Ruoslahti, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 7444 (2002).
35. We thank D. Hanahan for transgenic mice, R. M. Hoffman for the GFP-PC-3 cell line, M. J. Sailor and J.-H. Park for advice on the synthesis of iron oxide nanoworms, and E. Engvall for comments on the manuscript. This work was supported by grants CA104898, CA119414, CA119335, CA124427, CA115410, and CA30199 from the National Cancer Institute of the NIH and grants BC076050 and BC08544 from the U.S. Department of Defense. The authors and their institutions have patents on methods and compositions related to peptides and proteins with C-terminal elements and related to internalizing RGD peptides.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1183057/DC1
Materials and Methods
SOM Text
Figs. S1 to S16
Table S1
References

8 October 2009; accepted 25 March 2010
Published online 8 April 2010;
10.1126/science.1183057
Include this information when citing this paper.

Five-Vertebrate ChIP-seq Reveals the Evolutionary Dynamics of Transcription Factor Binding

Dominic Schmidt,^{1,2*} Michael D. Wilson,^{1,2*} Benoit Ballester,^{3*} Petra C. Schwalie,³ Gordon D. Brown,¹ Aileen Marshall,^{1,4} Claudia Kutter,¹ Stephen Watt,¹ Celia P. Martinez-Jimenez,⁵ Sarah Mackay,⁶ Iannis Talianidis,⁵ Paul Flicek,^{3,7†} Duncan T. Odom^{1,2†}

Transcription factors (TFs) direct gene expression by binding to DNA regulatory regions. To explore the evolution of gene regulation, we used chromatin immunoprecipitation with high-throughput sequencing (ChIP-seq) to determine experimentally the genome-wide occupancy of two TFs, CCAAT/enhancer-binding protein alpha and hepatocyte nuclear factor 4 alpha, in the livers of five vertebrates. Although each TF displays highly conserved DNA binding preferences, most binding is species-specific, and aligned binding events present in all five species are rare. Regions near genes with expression levels that are dependent on a TF are often bound by the TF in multiple species yet show no enhanced DNA sequence constraint. Binding divergence between species can be largely explained by sequence changes to the bound motifs. Among the binding events lost in one lineage, only half are recovered by another binding event within 10 kilobases. Our results reveal large interspecies differences in transcriptional regulation and provide insight into regulatory evolution.

The relationship between genetic sequence and transcriptional regulation is central to understanding species-specific biology, disease, and evolution (1). Identifying the divergence and conservation among functional regulatory ele-

ments is an important goal of comparative genomic research, and this is often done via DNA sequence comparisons using distant (2) and closely related species (3). Although both approaches have successfully identified conserved regulatory regions, the majority of transcription factor (TF) binding events can change rapidly between closely related species, making them difficult to detect using DNA sequence alone (4–7). For instance, the experimentally determined binding events for homologous TFs found in mouse and human livers are unlikely to align with each other (7), despite conservation of their functional targets (8) and global liver transcription (9). The evolution of mammalian transcriptional regulation remains largely unexplored beyond limited mouse-human comparisons.

We therefore identified the genome-wide binding of two TFs: (i) CCAAT/enhancer-binding protein alpha (CEBPA) in the livers of species

representing five vertebrate orders: human (primate), mouse (rodent), dog (carnivora), short-tailed opossum (didelphimorphia), and chicken (galliformes); and (ii) hepatocyte nuclear factor 4 alpha (HNF4A) in livers from humans, mice, and dogs. Chromatin immunoprecipitation experiments were combined with high-throughput sequencing (ChIP-seq) using healthy, nutritionally unstressed adult livers from the heterogametic sex as a functionally and transcriptionally conserved homologous tissue type (Fig. 1 and fig. S1) (8, 10).

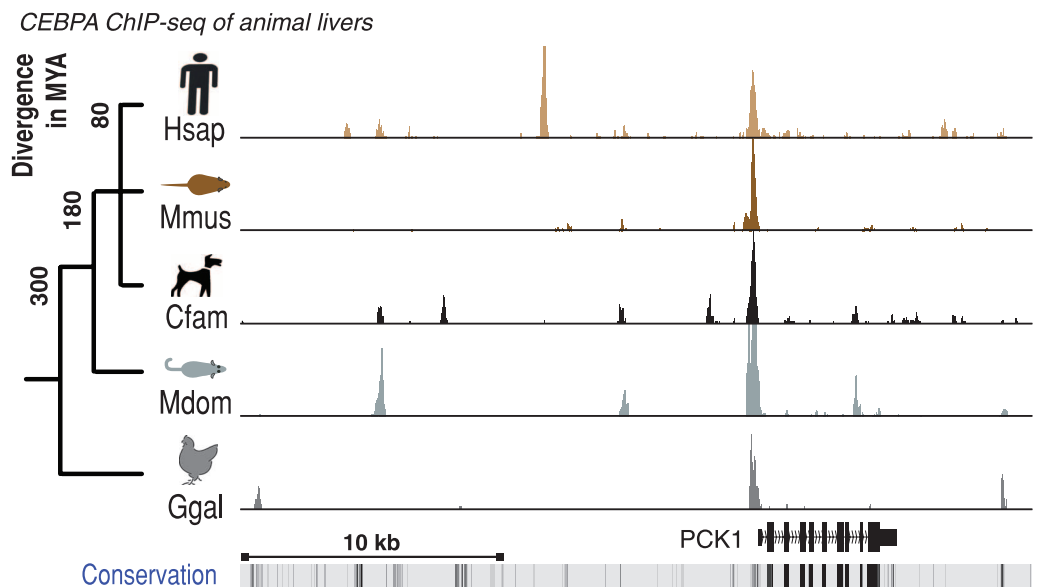
CEBPA and HNF4A were selected as representative TFs within the liver-specific regulatory network, because both are conserved and constitutively expressed with well-characterized target genes (10, 11). In addition, they represent distinct TF classes, and the DNA binding domains of each factor's orthologs are nearly identical among the study species (fig. S2).

The genomic TF occupancy data were reproducible between different individuals of the same species (fig. S3) and were validated by using alternative antibodies (fig. S4). Using a mouse carrying a human chromosome, we confirmed that genetic sequence, and not diet, lifestyle, or environment, is the primary determinant of liver-specific TF binding (fig. S5) (12). Given the greater evolutionary distance to opossum and chicken, contributions from non-genetic sources could be higher in those vertebrates.

We identified TF-bound regions using a dynamic programming algorithm, and our results were robust to different peak-calling thresholds (figs. S6 to S8) (13). To detect TF binding events shared among any combination of the five vertebrates, we used the Ensembl 12-way multispecies alignment (14), which incorporates approximately half of each species' genome into global alignments. Our findings did not substantially change with an alternate methodology that used pairwise alignments in a separate algorithm (figs. S6 to S8) (13).

Each TF bound between 16,000 and 30,000 locations in each mammalian genome; CEBPA bound approximately half this number in the smaller chicken

Fig. 1. CEBPA binding in vivo in livers isolated from five vertebrate species cross-mapped to the human *PCK1* gene locus. A rare ultraconserved binding event is shown surrounded by species-specific and partially shared binding events. On the left is the evolutionary tree of the five study species (Hsap, *Homo sapiens*; Mmus, *Mus musculus*; Cfam, *Canis familiaris*; Mdom, *Monodelphis domestica*; Ggal, *Gallus gallus*), with their approximate evolutionary distance in millions of years ago (MYA). The bottom track shows evolutionary conservation measured across 44 vertebrate species, and darker shading represents slower evolution.



genome (Fig. 2 and figs. S6, S7, and S9). For both factors, less than a quarter of bound regions were within 3 kb of known transcription start sites (TSSs). Between 30 and 50% of the binding sites of the two TFs overlapped in the genome (table S1). These overlapping sites did not exhibit substantially different characteristics in the conservation of underlying genetic sequence than the sites of CEBPA and HNF4A did when considered individually.

For these two liver-specific TFs, binding events appear to be shared 10 to 22% of the time between mammals from any two of the three placental

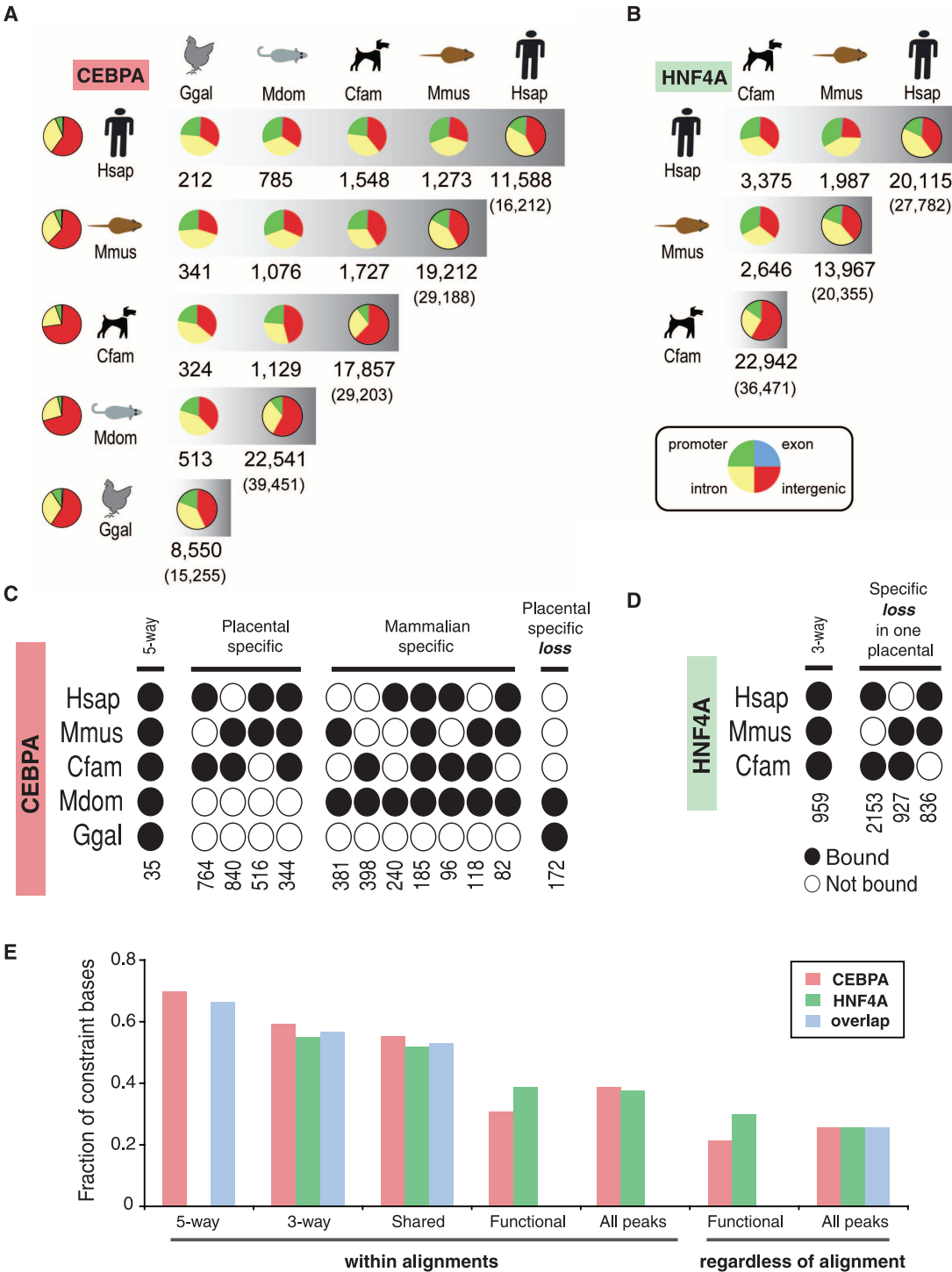
lineages we profiled, separated by approximately 80 million years of evolution (figs. S6 and S7). This result reveals a rapid rate of evolution in transcriptional regulation among closely related vertebrates. Nevertheless, the number of CEBPA and HNF4A TF binding events shared between any two of our five study species is far greater than could have occurred by chance (fig. S10).

We used the genome-wide binding of CEBPA in opossum to test the hypothesis that regulatory regions have diverged substantially between eutherian and metatherian mammals (15). Opossum

indeed showed dramatic changes in TF binding, and only between 6 and 8% of the genomic regions that are occupied by CEBPA in opossum liver align with CEBPA binding events also found in mouse, dog, and/or human livers. This divergence was even greater in chickens, which shared only 2% of CEBPA binding with humans, demonstrating extensive and continuous rewiring of gene regulation during vertebrate evolution that corresponds to evolutionary distance.

Ultraconserved noncoding regions are revealed by comparative genomic sequencing (16).

Fig. 2. Conservation and divergence of TF binding. For (A) CEBPA and (B) HNF4A, the pairwise distribution and numbers of binding events are shown as a pie chart distributed into the following segments: intergenic (red), intronic (yellow), exonic (blue), and promoter (TSS $\pm$ 3 kb) (green) regions. The left-most column contains the distributions of the bulk genomes. The right-most pie chart represents all binding events in each species, with the total number of alignable peaks above the total peaks (in parentheses). (C and D) Multispecies CEBPA and HNF4A binding event analysis, where black circles indicate binding in a given species. For instance, there are 764 regions bound by CEBPA only in dog and human (see also figs. S6, S7, and S17 and tables S2 and S6). (E) The DNA sequence constraint beneath binding events was measured by average GERP (20) scores for peaks found: in all five species (5-way), among all the placental mammals (3-way), bound in any two species (shared), within 10 kb of the TSS of functional targets (functional), and all peaks.



We identified ultrashared interactions between CEBPA and the vertebrate genome as binding events that were preserved over the 300 million years of evolution and thus were found in aligned positions in all five species: human, mouse, dog, opossum, and chicken. Using our most stringent

threshold, a set of 35 binding events were found to be shared by all five vertebrate species, and these binding events are almost invariably near genes that are central to liver-specific biology (Fig. 2C, tables S2 and S3). Although these ultrashared binding events are close to important liver-

specific genes, they make up less than 0.3% of the total CEBPA binding found in humans. About 250 direct functional HNF4A target genes have recently been identified by using multiple independent methodologies in mouse and human, including perturbation analysis in

Fig. 3. DNA binding specificities of CEBPA and HNF4A are highly conserved during vertebrate evolution. **(A)** The known sequence motifs were identified de novo in each species interrogated (13), and found within almost all binding events (fig. S12). **(B)** Multiple aligned motif occurrences are highly associated with binding events shared among three or more species. Peaks are categorized by the number of species in which they are shared, and the fraction of peaks with 0 (blue), 1 (gray), and 2 or more (red) aligned motifs are shown.

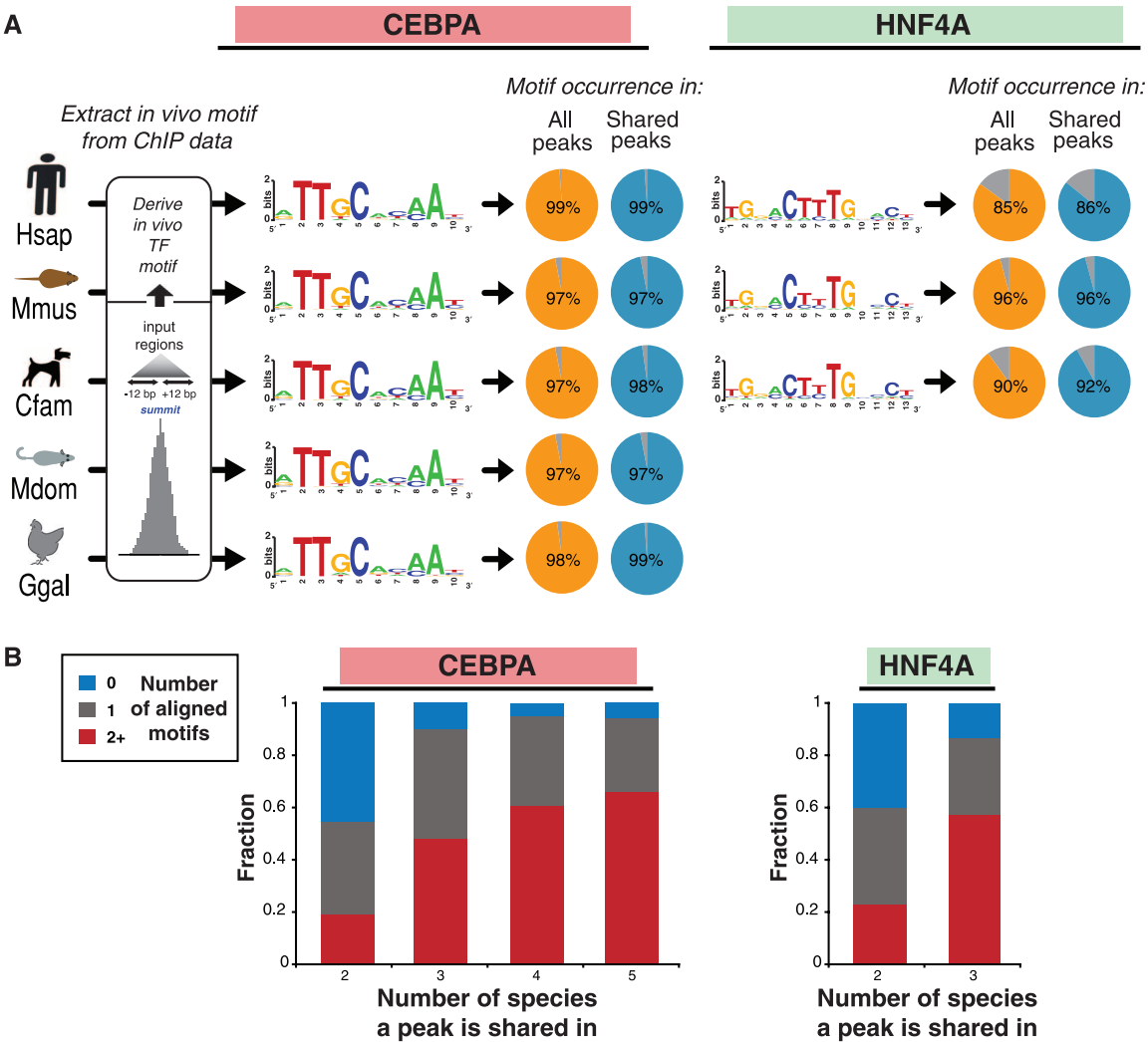
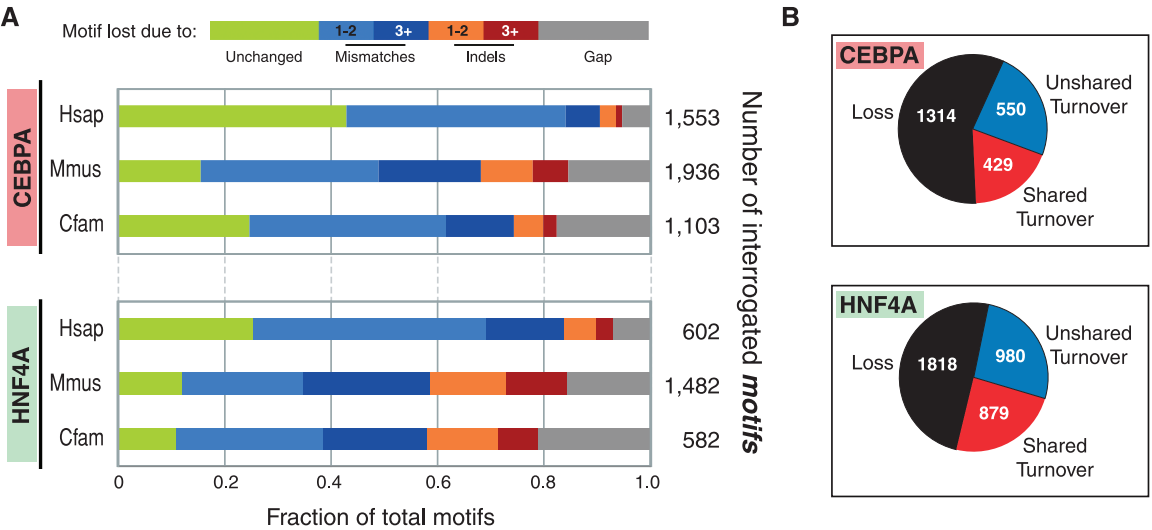


Fig. 4. Lineage-specific loss and turnover of TF binding events. **(A)** The unbound regions in each placental mammal that align to regions showing TF binding in the other two placental mammals were collected, and the mechanisms by which the underlying motifs were disrupted were summarized. **(B)** Turnovers occurred near lineage-specific lost binding events approximately half the time; shared turnovers represent cases where a cluster of binding events likely occurred in a common ancestor (fig. S16).



both species (8). We experimentally identified a similar set of transcriptional target genes whose expression is dependent on CEBPA in adult mouse livers by using a conditional knock-out strategy (17). In mammals, the target genes for both TFs have a disproportionate fraction of binding events that are shared in at least two species (P value $< 1 \times 10^{-5}$) (table S4). CEBPA binding near direct target genes did not overlap with the binding events shared by five species.

We further compared our results to a set of 53 regulatory sequences within known, authentic liver enhancers in humans (table S5) (18). Thirty-eight of these regulatory sequences were located within nine HNF4A-bound regions. CEBPA binding overlapped with five of these HNF4A-bound regions, and we also found that five of the nine HNF4A binding events were bound by HNF4A in more than one species. Overall, these findings suggest that functional targets are enriched for TF binding events found in multiple species.

Mammalian TF binding studies have suggested that functional enhancers show increased sequence constraint (19). As expected, the relatively few binding events shared among three or five species showed increased sequence constraint. The sequence constraint, which was evaluated by genomic evolutionary rate profiling (GERP) scores (20), in bound regions near functional targets was similar to that for all bound regions for both TFs, and these results were robust to the method applied. Regions bound by both CEBPA and HNF4A have sequence constraint patterns similar to those found for each factor analyzed independently (Fig. 2E and fig. S11). In sum, TF binding events near functional targets showed enhanced sharing between species, without a corresponding increase in sequence constraint.

DNA binding specificities of TFs show remarkable diversity and complexity (21), yet few studies have compared specificities of orthologous TFs among multiple species. The motifs we directly determined from experimental binding data showed that in vivo bound consensus sequences remained virtually unchanged during vertebrate evolution, despite most binding events being species-specific (Fig. 3A and fig. S12). Neither the quality of a bound motif, as determined by its similarity to the consensus, nor the regional ChIP enrichment, as measured by sequencing read depth, was correlated with the conservation of TF binding events (fig. S13).

Searching for the sequence features that are associated with shared binding events, we discovered that binding events shared by more species contain more aligned motifs (Fig. 4B). These shared regions represent examples of deeply conserved regulatory architecture featuring multiple motifs at specific sequence locations maintained through vertebrate evolution. The most conserved of these, the five-way ultrashared sites, also exhibit the strongest sequence constraint (Fig. 2E).

To explore the genetic mechanisms underlying the divergence of TF binding, we identified potentially lost CEBPA and HNF4A binding

events. A binding event was assumed to be lost if it was not present in one placental mammal yet was experimentally found at aligned, orthologous regions in the other two placental mammals. Using parsimony, this situation is best explained by an ancestral TF binding event present before the mammalian radiation that was subsequently lost along one lineage.

The lost binding events were categorized by the sequence changes to the alignable binding motifs within the orthologous regions of the other species (Fig. 4). Between 20 and 40% of the motifs associated with lineage-specific binding event losses were unchanged. These regions may represent cases of epigenetic redirection, yet-to-be characterized single-nucleotide polymorphisms (SNPs) or indels, or loss of nearby genomic binding partners. A larger fraction of the absent binding events was associated with motifs whose disruption could be assigned to base pair substitutions, indels, and gaps in the alignment. Across all the vertebrate species, indels appear to be associated with loss of the underlying sequence motif a third as often as mismatches. A four-mammal analysis, using opossum as an outgroup, afforded similar results (fig. S14). Analogous mechanisms appear to explain species-specific gains of TF binding events (fig. S15). Taken together, the steady accumulation of small changes in the genetic sequence appears to rapidly remodel thousands of TF binding sites in mammals.

Approximately half of lineage-specific losses of TF binding showed evidence of nearby compensatory binding events (Fig. 4B). A quarter of species-specific losses had a nearby (± 10 kb) gained binding event that is unique to the same lineage (unshared turnover), and an additional quarter of the losses had a nearby binding event that is shared in one or more other species (shared turnover) (fig. S16). The latter case suggests the existence of a cluster of binding events in the common ancestor. In both cases, the probability of finding a turnover decreased rapidly with distance from the loss (fig. S16), but a shared turnover was typically closer to the site of the loss than was an unshared turnover [P value $< 1.0 \times 10^{-10}$ (CEBPA) and P value $< 1 \times 10^{-15}$ (HNF4A)].

Understanding the evolutionary dynamics of TF binding is essential to understanding the evolution of gene regulation. Many comparative genomics approaches assume that a multispecies alignment of a high-quality motif is indicative of functionality (20, 22–27). Our analysis of experimentally determined in vivo occupancy of two TFs in multiple vertebrates revealed apparent limitations to this model and a number of other insights about the complex relationship between genetic sequence, TF binding, and genome regulation.

First, the vast majority of ChIP-identified TF binding events are unique to each vertebrate species; in mammals, the binding events that occur within species-specific, repetitive DNA are more common than conserved binding events. Sec-

ond, ultrashared TF binding events, which are the functional counterpart of ultraconserved sequences, appear rarely in vivo among all five vertebrates. Third, only approximately half of the binding events that are lost in one placental mammal yet present in at least two others are potentially recovered by nearby turnover events. Fourth, neither motif nor strength of TF binding correlate with conservation of a TF's genomic occupancy. Alterations in the DNA binding specificity of CEBPA and HNF4A cannot account for rapid binding divergence, nor can species-specific environmental differences (12).

Nevertheless, comparing binding events within 10 kb of the TSS of experimentally determined target genes of CEBPA and HNF4A has shown that binding events near these genes are more likely to be shared with other species, although this does not correspond to an increase in sequence constraint. In fact, the set of the ultrashared, five-way binding events is entirely disjoint from the set of genes that are directly dependent on CEBPA in adult liver. For HNF4A, only 6% of binding events shared across three placental mammals (Fig. 2D) are near the highest-quality functional target genes, namely, those genes that depend on HNF4A for proper expression in both mouse and human. Given that most TFs are active in multiple cell types (28), it is possible that the remaining shared sites are active in other tissues or other developmental stages. Indeed, the ultrashared CEBPA binding events are uniformly found near liver-specific genes that would be expected to be up-regulated upon liver organogenesis. Conversely, those binding events near functional targets in adult liver that are neither shared nor show signs of sequence constraint may represent lineage-specific regulatory interactions.

The preponderance of species-specific binding and the rapid lineage-specific loss of binding events suggests that a sizeable majority of specific TF-DNA interactions could be evolving neutrally. Liver-specific TFs and subsequent gene expression are both highly conserved; the rapid gain and loss of binding events may be indicative of compensatory changes that maintain local concentrations of TF binding near functional targets (29). Indeed, a recent computational approach that uses a high concentration of TF binding motifs, regardless of their alignment, showed improved ability to predict regulatory interactions (30).

Despite the rapid gain and loss of TF binding events in mammals, tissue-specific gene regulation seems to be maintained by identifiable regulatory architectures that can be independent of sequence constraint.

References and Notes

1. G. A. Wray, *Nat. Rev. Genet.* **8**, 206 (2007).
2. B. Lenhard et al., *J. Biol.* **2**, 13 (2003).
3. D. Boffelli et al., *Science* **299**, 1391 (2003).
4. A. R. Borneman et al., *Science* **317**, 815 (2007).
5. E. T. Dermitzakis, A. G. Clark, *Mol. Biol. Evol.* **19**, 1114 (2002).

6. ENCODE Project Consortium, *Nature* **447**, 799 (2007).
7. D. T. Odom *et al.*, *Nat. Genet.* **39**, 730 (2007).
8. S. F. Boj *et al.*, *Diabetes* **58**, 1245 (2009).
9. E. T. Chan *et al.*, *J. Biol.* **8**, 33 (2009).
10. C. P. Martinez-Jimenez, I. Kyrmizi, P. Cardot, F. J. Gonzalez, I. Talianidis, *Mol. Cell. Biol.* **30**, 656 (2010).
11. P. Hatzis, I. Kyrmizi, I. Talianidis, *Mol. Cell. Biol.* **26**, 7017 (2006).
12. M. D. Wilson *et al.*, *Science* **322**, 434 (2008).
13. Materials and methods are available as supporting material on Science Online.
14. T. J. Hubbard *et al.*, *Nucleic Acids Res.* **37** (Database issue), D690 (2009).
15. T. S. Mikkelsen *et al.*, *Nature* **447**, 167 (2007).
16. G. Bejerano *et al.*, *Science* **304**, 1321 (2004).
17. Y. H. Lee, B. Sauer, P. F. Johnson, F. J. Gonzalez, *Mol. Cell. Biol.* **17**, 6014 (1997).
18. E. Portales-Casamar *et al.*, *Genome Biol.* **8**, R207 (2007).
19. A. Visel *et al.*, *Nature* **457**, 854 (2009).
20. G. M. Cooper *et al.*, *Genome Res.* **15**, 901 (2005).
21. G. Badis *et al.*, *Science* **324**, 1720 (2009).
22. E. H. Margulies *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 4795 (2005).
23. M. Blanchette *et al.*, *Genome Res.* **16**, 656 (2006).
24. J. Taylor *et al.*, *Genome Res.* **16**, 1596 (2006).
25. E. H. Margulies *et al.*, *Genome Res.* **17**, 760 (2007).
26. W. Miller *et al.*, *Genome Res.* **17**, 1797 (2007).
27. X. Xie *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 7145 (2007).
28. J. M. Vaquerizas, S. K. Kummerfeld, S. A. Teichmann, N. M. Luscombe, *Nat. Rev. Genet.* **10**, 252 (2009).
29. S. MacArthur *et al.*, *Genome Biol.* **10**, R80 (2009).
30. R. Gordân, L. Narlikar, A. J. Hartemink, *Nucleic Acids Res.* **38**, e90 (2010).
31. We thank N. Matthews and J. Hadfield at the Cambridge Research Institute (CRI) Genomics Core; the CRI Bioinformatics Core; W. Howat and the Histopathology Core; T. Davidge, S. Ballantyne (CRI); A. Enright, S. Wilder, and J. Herrero (European Bioinformatics Institute). This work was supported by the European Research Council Starting Grant, the European Molecular Biology Organization Young Investigator Award, Addenbrooke's Biomedical Research Centre, Hutchinson Whampoa (D.T.O.); Swiss National Science Foundation (C.K.); University of Cambridge (D.S., M.D.W., and D.T.O.); Cancer Research UK (D.S., M.D.W., G.B., C.K., and D.T.O.); the Wellcome Trust (grant nos. WT062023 and WT079643) (B.B. and P.F.); and European Molecular Biology Laboratory (P.C.S. and P.F.). ChIP-seq experiments were deposited into ArrayExpress under the accession number E-TABM-722. CEBPA knockout gene expression experiments were deposited into ArrayExpress under the accession number E-MTAB-178. Author contributions: D.S., M.D.W., and D.T.O. designed experiments; D.S., M.D.W., C.K., S.W., and C.P.M.-J. performed experiments; D.S., M.D.W., B.B., G.B., P.C.S., and P.F. analyzed the data; S.M., C.P.M.-J., I.T., and A.M. provided tissues; D.S., M.D.W., B.B., I.T., P.C.S., P.F., and D.T.O. wrote the manuscript. P.F. and D.T.O. oversaw the work.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1186176/DC1

Materials and Methods

Figs. S1 to S17

Tables S1 to S7

References

21 December 2009; accepted 24 March 2010

Published online 8 April 2010;

10.1126/science.1186176

Include this information when citing this paper.

pH Sensing by Intracellular *Salmonella* Induces Effector Translocation

Xiu-Jun Yu, Kieran McGourty, Mei Liu, Kate E. Unsworth,* David W. Holden†

Salmonella enterica is an important intracellular bacterial pathogen of humans and animals. It replicates within host-cell vacuoles by delivering virulence (effector) proteins through a vacuolar membrane pore made by the *Salmonella* pathogenicity island 2 (SPI-2) type III secretion system (T3SS). T3SS assembly follows vacuole acidification, but when bacteria are grown at low pH, effector secretion is negligible. We found that effector secretion was activated at low pH from mutant strains lacking a complex of SPI-2–encoded proteins SsaM, SpiC, and SsaL. Exposure of wild-type bacteria to pH 7.2 after growth at pH 5.0 caused dissociation and degradation of SsaM/SpiC/SsaL complexes and effector secretion. In infected cells, loss of the pH 7.2 signal through acidification of host-cell cytosol prevented complex degradation and effector translocation. Thus, intravacuolar *Salmonella* senses host cytosolic pH, resulting in the degradation of regulatory complex proteins and effector translocation.

Type III secretion systems (T3SSs) are assembled in the cell envelope of many Gram-negative bacterial pathogens. They secrete three classes of proteins: subunits of a surface-exposed needle-like structure; translocon proteins, which form a pore in host membranes; and effectors, which pass through the needle channel and translocon pore into the host cell, where they manipulate cellular processes and promote bacterial virulence (1). Translocon pore assembly must precede effector translocation, and it is thought that upon assembly of the pore, bacteria sense host cell signals to activate effector secretion. The physiological signals and mechanisms underlying transition from translocon to effector secretion are not understood.

Assembly of the *Salmonella* pathogenicity island 2 (SPI-2) T3SS in vivo occurs after acidification of *Salmonella*-containing vacuoles (SCVs) to pH ~5.0 (Fig. 1A) (2, 3). Bacteria grown in vitro at pH 5.0 secrete translocon proteins but negligible levels of effectors (4). SPI-2–encoded SsaL is a member of the YopN/InvE/MxiC/SepL family of regulatory proteins found throughout T3SSs of animal pathogens (5) and is required for secretion of the translocon proteins SseB and SseD (6). To study regulation of the translocon-to-effector switch, we examined protein secretion from a *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) ssaL mutant after growth at pH 5.0 in vitro (7). This strain displayed enhanced secretion of chromosome-expressed double hemagglutinin (2HA)–tagged SPI-2 T3SS effector SseJ (Fig. 1B) and chromosome or plasmid-expressed SseF (fig. S1) and did not secrete translocon proteins SseB or SseC (Fig. 1, B and C). The secretion pattern for SseJ-2HA and translocon proteins was restored to wild type by introduction of a plasmid expressing SsaL-2HA (Fig. 1C). There was no detectable secretion of SseB or ef-

factors by an ssaV mutant strain, which has a nonfunctional SPI-2 T3SS (Fig. 1B). The ssaL mutant phenotype is very similar to that of strains lacking SPI-2–encoded SpiC or SsaM (Fig. 1B) (4). Thus SsaL, SsaM, and SpiC are all required for secretion of translocon proteins and suppress the secretion of effectors under conditions that simulate the vacuolar environment (Fig. 1A).

SpiC and SsaM form a complex (4). To determine whether SsaL interacts with the SpiC/SsaM complex when produced at physiological levels, we constructed a bacterial strain in which chromosomal copies of spiC, ssaM, and ssaL were replaced with versions expressing epitope-tagged proteins (SpiC-2HA, T7-SsaM, and SsaL-3Flag). This strain (wt-3tag) was indistinguishable from the wild-type strain in terms of intracellular replication and SPI-2 T3SS effector-dependent tubule formation in infected cells, indicating a functional T3SS (fig. S2). The wt-3tag strain and an isogenic mutant lacking endogenous or epitope-tagged SpiC (spiC) were grown at pH 5.0, and whole-cell lysates were immunoprecipitated with an antibody to T7. SsaL-3Flag was coprecipitated from the wt-3tag strain but not from the spiC mutant (Fig. 1D). Co-immunoprecipitation and pull-down experiments also showed that the C-terminal 18 amino acids of SsaM were required for interaction with SpiC (4) and SsaL (Fig. 2A). Thus, SsaL, SsaM, and SpiC form a complex when bacteria are grown at pH 5.0 (Fig. 1A).

To determine whether an intact complex is necessary to suppress effector secretion at pH 5.0, we constructed three small deletion mutants in an N-terminal region of SsaL corresponding to the chaperone-binding domain of YopN (fig. S3), which is necessary for its interaction with SycN and YscB (8). These mutants, and a truncated SsaM protein lacking its 18 C-terminal amino acids, all prevented formation of the ternary complex (Fig. 2, A and B) and caused the same phenotype as individual null mutants: no detectable translocon secretion and greatly enhanced effector secretion at pH 5.0 (Fig. 2, C and D).

Section of Microbiology, Centre for Molecular Microbiology and Infection, Imperial College London, Armstrong Road, London SW7 2AZ, UK.

*Present address: GeoMed, St Peter's Working Institute, Windmill Street, Macclesfield, Cheshire SK11 7HS, UK.

†To whom correspondence should be addressed. E-mail: d.holden@imperial.ac.uk

Thus, an intact ternary complex is necessary to promote translocon secretion and to suppress effector secretion at pH 5.0.

The *ssaM*, *spiC*, and *ssaL* mutant phenotypes raised the possibility that their corresponding proteins might regulate the secretion switch in infected cells once the translocon pore has been assembled. The translocon spans the membrane separating the vacuole lumen (pH ~5.0) (9) from the cytosol (pH 7.2). We tested whether a shift in ambient pH from 5.0 to 7.2 enhanced SPI-2 effector secretion by using wild-type bacteria in vitro. Bacterial strains expressing either SseJ-2HA or SseF-2HA were grown at pH 5.0 for 4 hours in order to activate the SPI-2 T3SS and then exposed to pH 7.2 or 5.0. Secretion of these and other effectors was enhanced by an order of magnitude when wild-type bacteria were subjected to pH shift (Fig. 3A and fig. S4A). The overall amounts of each secreted protein were similar to those secreted by the *spiC* mutant strain at pH 5.0 or after shift to pH 7.2, and there was no detectable secretion by the *ssaV* mutant strain (Fig. 3A). In contrast, intrabacterial and secreted levels of SseB were reduced by approximately 50% after pH shift (fig. S4A). Inhibition of protein synthesis showed that the pH shift enhanced the secretion of preformed effectors, suggesting that it acted on the T3SS apparatus or associated proteins (fig. S4B). Enhanced secretion of SseF-2HA was detected at pH 6.4 and reached maximal levels at pH 6.8 (fig. S4C). Thus, wild-type bacteria respond to an extracellular pH shift from 5.0 to 7.2 by reducing SseB levels and triggering effector secretion.

To determine whether host cytosolic pH (pH_{cyt}) acted as a signal for the secretion switch, digitonin was used to selectively permeabilize plasma membranes of infected epithelial cells exposed to external media at different pH (pHe). After digitonin permeabilization, pH_{cyt} was reduced by pHe 6.0, but SCV luminal pH remained within the normal range (fig. S5). Secreted SseB was detected at similar levels in untreated and permeabilized cells at pHe 6.0 (Fig. 3B). Translocation of SseF-2HA was detected in over 90% of infected permeabilized cells at pHe 7.2 (Fig. 3C) but in less than 13% of infected cells at pHe 6.0 (Fig. 3C). This inhibition was reversed if pHe was subsequently raised to 7.2 (Fig. 3C). Thus, we have observed a pH-dependent, reversible inhibition of effector translocation, which suggests that near-neutral host cytosolic pH is necessary for effector delivery across the vacuolar membrane.

We next investigated SsaL/SsaM/SpiC complex integrity in bacteria after pH shift. The wt-3tag strain was grown at pH 5.0 for 4 hours and then exposed to pH 7.2 for 1 hour. Because substrate recognition in T3SSs involves proteins associated with the secretion machinery in the bacterial inner membrane (1), bacterial cells were lysed and separated into membrane and cytosolic fractions. Membrane-associated SpiC-2HA was immunoprecipitated and immunoblots probed

so as to detect SsaL-3Flag and T7-SsaM. As expected, both proteins were co-precipitated at pH 5.0, but neither was detected when SpiC-2HA

was precipitated from membranes of cells shifted to pH 7.2 (Fig. 4A). When T7-SsaM was immunoprecipitated from membranes recovered from

Fig. 1. SsaL is required for translocon protein secretion, suppresses effector secretion, and interacts with SsaM and SpiC. (A) Model of the SPI-2 T3SS spanning the inner and outer membranes of the bacterial cell and connected to a translocon pore formed in the vacuolar membrane. Translocon proteins must be secreted before effectors can be translocated. SsaV is thought to be located in the inner membrane and is essential for the function of the secretion system. **(B)** Wild-type (wt), *ssaV*, *spiC*, *ssaM*, or *ssaL* deletion mutant strains expressing 2HA-tagged SseJ from chromosome were grown in minimal medium pH 5.0, and secreted and bacterial-associated (lysate) proteins were examined by means of immunoblotting to detect the HA epitope, SseB, and DnaK. **(C)** A plasmid expressing SsaL-2HA was introduced into the *ssaL* deletion mutant, and secreted levels of SseJ-2HA, SseB, and SseC were compared with the *ssaL* mutant by means of immunoblotting. **(D)** Interaction between SpiC-2HA, T7-SsaM, and SsaL-3Flag. In the wt-3tag strain, *spiC*, *ssaM*, and *ssaL* are replaced with versions expressing epitope-tagged proteins. This and an isogenic strain lacking SpiC (*spiC*) were grown in minimal medium pH 5.0, and whole-cell lysates were immunoprecipitated with an antibody to T7. The presence of the three proteins was detected in input samples (input) and after immunoprecipitation (output) by means of immunoblotting.

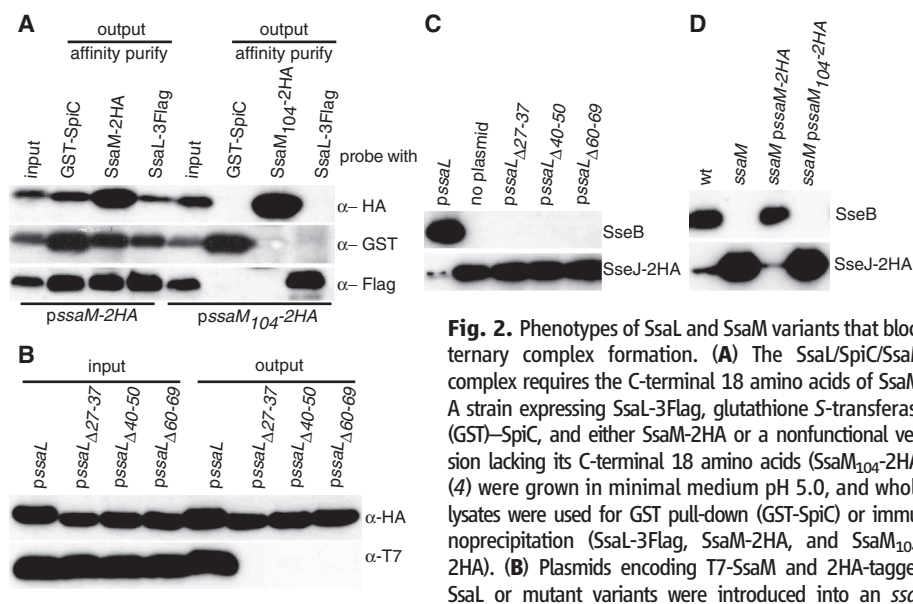
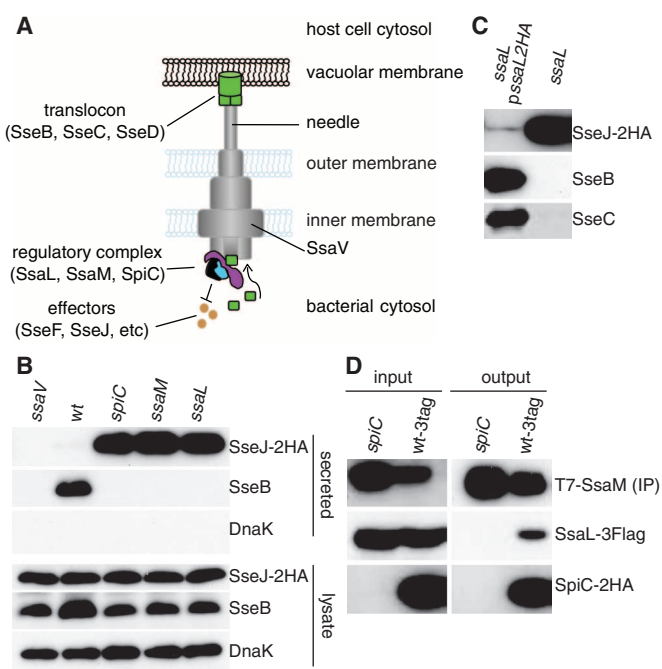


Fig. 2. Phenotypes of SsaL and SsaM variants that block ternary complex formation. (A) The SsaL/SpiC/SsaM complex requires the C-terminal 18 amino acids of SsaM. A strain expressing SsaL-3Flag, glutathione S-transferase (GST)-SpiC, and either SsaM-2HA or a nonfunctional version lacking its C-terminal 18 amino acids (SsaM₁₀₄-2HA) (4) were grown in minimal medium pH 5.0, and whole lysates were used for GST pull-down (GST-SpiC) or immunoprecipitation (SsaL-3Flag, SsaM-2HA, and SsaM₁₀₄-2HA). **(B)** Plasmids encoding T7-SsaM and 2HA-tagged SsaL or mutant variants were introduced into an *ssaL* deletion mutant. Whole bacterial lysates were immunoprecipitated with antibody to HA. SsaL-2HA and T7-SsaM were detected in input samples (input) and after immunoprecipitation (output) by means of immunoblotting. **(C)** The *ssaL* deletion strain expressing SseB and SseJ-2HA, and SsaL or mutant variants from a plasmid, were grown in minimal medium pH 5.0 for 5 hours. Secreted fractions were analyzed by means of immunoblotting for SseB and SseJ-2HA. **(D)** The wild-type strain, an *ssaM* mutant, and the mutant with or without a plasmid expressing SsaM-2HA or SsaM₁₀₄-2HA were grown at pH 5.0 and analyzed as in (C).

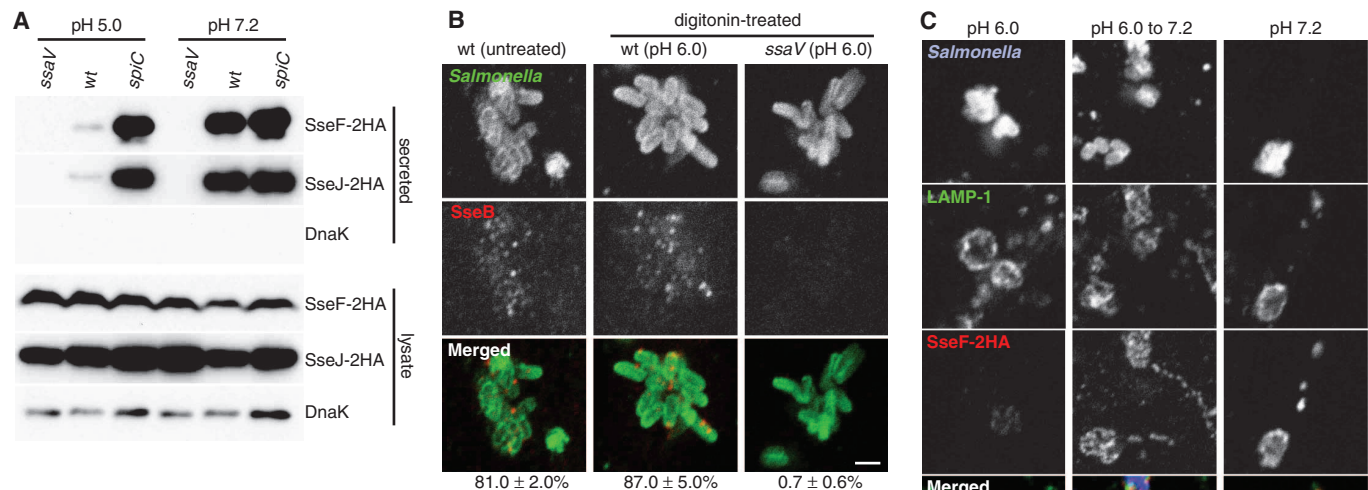


Fig. 3. Effect of pH on effector secretion and translocation. **(A)** Bacterial strains were grown in minimal medium pH 5.0 for 4 hours, then exposed to pH 5.0 or 7.2 for 90 min. Secreted and bacteria-associated (lysate) 2HA-tagged effectors and DnaK were examined by means of immunoblotting. **(B)** HeLa cells were infected with wild-type or *ssaV* mutant *Salmonella* for 3.5 hours in order to allow expression of the SPI-2 T3SS, then some samples were permeabilized with digitonin and exposed to pHe 6.0. Cells were fixed 2.5 hours later and immunolabeled in order to detect *Salmonella* and secreted SseB. **(C)** HeLa cells were infected for 3.5 hours with wild-type *Salmonella* expressing SseF-2HA, then permeabilized with digitonin and exposed to pH 6.0 or 7.2 for a further 2.5 hours. In one sample, pHe was changed from 6.0 to 7.2, 1 hour before fixation. Fixed cells were immunolabeled to detect *Salmonella*, LAMP-1, and SseF-2HA. In **(B)** and **(C)**, values below the images represent the percentage of cells in which secreted SseB or translocated SseF-2HA was detected, ±SE of three experiments ($n > 100$ cells per experiment). Scale bars, 2 μ m.

bacteria at pH 5.0, both SsaL-3Flag and SpiC-2HA were co-precipitated, but not after shift of bacteria to pH 7.2 (Fig. 4A). Thus, an increase in extracellular pH leads to dissociation of the membrane-bound SsaL/SsaM/SpiC complex.

We were unable to detect secretion or translocation of SsaM, SpiC (4), or SsaL (fig. S6). To study the stability of SsaL and SsaM after pH shift, the wt-3tag strain grown at pH 5.0 was exposed to either pH 5.0 or pH 7.2 in the presence of tetracycline in order to inhibit further protein synthesis, and protein levels were examined. Whereas the levels of DnaK and a functional 3Flag-tagged SsaN (an essential component of the SPI-2 T3SS) were relatively stable over this period at either pH, levels of both SsaL-3Flag and T7-SsaM declined. However, the rate of decline was much greater in bacteria after exposure to pH 7.2 as compared with that after exposure to pH 5.0 (Fig. 4B). To determine whether intrabacterial levels of SsaL and SsaM were affected after translocon pore assembly in vivo, HeLa cells were infected for 6 hours with the wt-3tag strain or by a mutant version lacking all three translocon proteins. The levels of SsaL-3Flag and T7-SsaM in intracellular wt-3tag bacteria were consistently lower (by $36.7 \pm 1.3\%$ and $33.7 \pm 3.6\%$, respectively) as compared with their levels in the strain that could not assemble a translocon pore (Fig. 4C) but had an otherwise functional T3SS (fig. S7). Furthermore, loss of SsaL-3Flag and T7-SsaM was prevented by reducing pHcyt and induced after a subsequent increase in pHcyt (causing a loss of $57.3 \pm 0.6\%$ and $60.2 \pm 1.9\%$, respectively) (Fig. 4C). Thus, in infected cells, loss of SsaL and SsaM requires

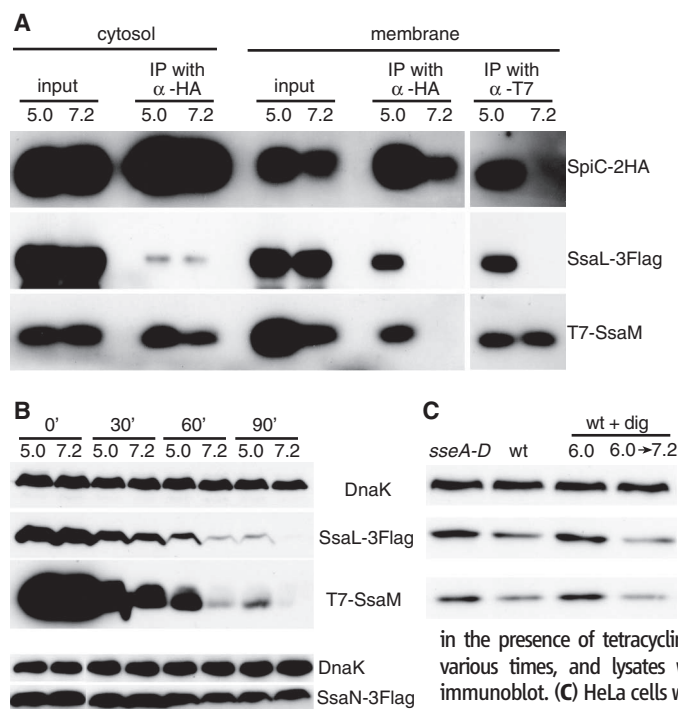


Fig. 4. Effect of pH on the SsaL/SsaM/SpiC complex. **(A)** The wt-3tag strain was grown in minimal medium at pH 5.0 for 4 hours, then exposed to pH 5.0 or 7.2 for 1 hour, then membrane-associated and cytosolic SpiC-2HA and membrane-associated T7-SsaM were immunoprecipitated (IP). Proteins were detected in input samples (input) and after immunoprecipitation (output) by means of immunoblotting. **(B)** The wt-3tag strain and a strain in which the chromosomal copy of *ssaV* is replaced with a functional version carrying a 3Flag epitope (bottom) were subjected to pH shift in the presence of tetracycline. Samples were removed at various times, and lysates were analyzed by means of immunoblot. **(C)** HeLa cells were infected with the wt-3tag strain (wt) or an isogenic translocon mutant (*ssaA-D*). At 3.5 hours after invasion, some wt-infected cells were treated with digitonin (wt + dig), and exposed to pHe 6.0 for 2.5 hours (6.0) or pHe 6.0 for 1.5 hours, then changed to pHe 7.2 for another 1 hour (6.0 $\rightarrow$ 7.2). Cells were lysed at 6 hours after invasion and analyzed by means of immunoblotting.

near-neutral pHcyt and formation of the translocon. The translocon pore provides a means by which intravacuolar bacteria could sense pHcyt, leading to dissociation and degradation of regulatory complex components, triggering effector delivery (fig. S8).

Acidic ambient pH suppresses effector secretion but is necessary for translocon protein secretion (3, 4, 10). The SsaL/SsaM/SpiC regulatory complex has a critical role in this process by acting as a “gatekeeper,” enabling translocon protein secretion while suppressing effector secretion

at pH 5.0. This complex is likely to interact with the cytoplasmic region of basal body of the secretion apparatus and to respond to an unidentified pH sensor. The sensor is unlikely to be part of the translocon because the translocon deletion mutant displayed wild-type levels of effector secretion upon pH upshift (fig. S7). The sensor might be the needle subunit itself, which has been implicated in signaling the translocator to effector switch in *Shigella* (11) and Yop secretion by *Yersinia* (12). Another possibility is that translocon pore assembly changes the pH gradient within the needle channel and that the sensor is located toward the base of the secretion apparatus. Changes in pH from mildly acidic to neutral can have dramatic effects on protein folding; for example, some bacterial toxins refold after their translocation from acidic endosomes to the host-cell cytosol in a partially un-

folded state (13). The SPI-2 T3SS pH sensor might thus undergo a conformational change on exposure to neutral pH and transduce a dissociation signal to the SsaL/SsaM/SpiC complex.

References and Notes

1. J. E. Galán, H. Wolf-Watz, *Nature* **444**, 567 (2006).
2. C. Rappl, J. Deiwick, M. Hensel, *FEMS Microbiol. Lett.* **226**, 363 (2003).
3. D. Chakravorty, M. Rohde, L. Jäger, J. Deiwick, M. Hensel, *EMBO J.* **24**, 2043 (2005).
4. X.-J. Yu, M. Liu, D. W. Holden, *Mol. Microbiol.* **54**, 604 (2004).
5. M. J. Pallen, S. A. Beatson, C. M. Bailey, *BMC Microbiol.* **5**, 9 (2005).
6. B. K. Coombes, N. F. Brown, Y. Valdez, J. H. Brumell, B. B. Finlay, *J. Biol. Chem.* **279**, 49804 (2004).
7. Materials and methods are available as supporting material on Science Online.
8. F. D. Schubot *et al.*, *J. Mol. Biol.* **346**, 1147 (2005).
9. D. Drecktrah, L. A. Knodler, D. Howe, O. Steele-Mortimer, *Traffic* **8**, 212 (2007).

10. C. R. Beuzón, G. Banks, J. Deiwick, M. Hensel, D. W. Holden, *Mol. Microbiol.* **33**, 806 (1999).
11. R. Kenjale *et al.*, *J. Biol. Chem.* **280**, 42929 (2005).
12. J. Torruellas, M. W. Jackson, J. W. Pennock, G. V. Plano, *Mol. Microbiol.* **57**, 1719 (2005).
13. J. A. Young, R. J. Collier, *Annu. Rev. Biochem.* **76**, 243 (2007).
14. We thank J. Mota, C. Tang, and members of the Holden laboratory for comments on the manuscript. This research was supported by grants G0800148 and 074553/Z/04/Z to D.W.H. from the Medical Research Council and Wellcome Trust.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1189000/DC1
Materials and Methods
Figs. S1 to S8
References

2 March 2010; accepted 25 March 2010

Published online 15 April 2010;

10.1126/science.1189000

Include this information when citing this paper.

A Global Protein Kinase and Phosphatase Interaction Network in Yeast

Ashton Breitkreutz,^{1*} Hyungwon Choi,^{2*} Jeffrey R. Sharom,^{1,3*} Lorrie Boucher,^{1*} Victor Neduva,^{4*} Brett Larsen,¹ Zhen-Yuan Lin,¹ Bobby-Joe Breitkreutz,¹ Chris Stark,¹ Guomin Liu,¹ Jessica Ahn,¹ Danielle Dewar-Darch,¹ Teresa Reguly,¹ Xiaojing Tang,¹ Ricardo Almeida,⁴ Zhaohui Steve Qin,⁵ Tony Pawson,^{1,3} Anne-Claude Gingras,^{1,3,†} Alexey I. Nesvizhskii,^{2,6,†} Mike Tyers^{1,3,4,†}

The interactions of protein kinases and phosphatases with their regulatory subunits and substrates underpin cellular regulation. We identified a kinase and phosphatase interaction (KPI) network of 1844 interactions in budding yeast by mass spectrometric analysis of protein complexes. The KPI network contained many dense local regions of interactions that suggested new functions. Notably, the cell cycle phosphatase Cdc14 associated with multiple kinases that revealed roles for Cdc14 in mitogen-activated protein kinase signaling, the DNA damage response, and metabolism, whereas interactions of the target of rapamycin complex 1 (TORC1) uncovered new effector kinases in nitrogen and carbon metabolism. An extensive backbone of kinase-kinase interactions cross-connects the proteome and may serve to coordinate diverse cellular responses.

Protein phosphorylation mediates cellular responses to growth factors, environmental signals, and internal processes by the regulation of protein interactions, enzyme activity, or protein localization (1). However, the protein interactions of kinases, phosphatases, and their regulatory subunits and substrates remain sparse-

ly mapped, particularly in high-throughput (HTP) datasets [fig. S1 (2)]. To chart the budding yeast kinase and phosphatase interaction (KPI) network, we systematically characterized protein kinase and phosphatase complexes by rapid magnetic bead capture, on-bead protein digestion, and mass spectrometric identification of associated proteins, using different epitope tags and expression systems [fig. S2; (2)]. One hundred thirty protein kinases, 24 lipid and metabolic kinases, 47 kinase regulatory subunits, 38 protein phosphatases, 32 phosphatase regulatory subunits, and 5 metabolic phosphatases were analyzed (tables S1 and S2).

We eliminated nonspecific interactions using a statistical model called Significance Analysis of Interactome (SAINT). In contrast to simple threshold models, SAINT assigns the number of peptide identifications for each interactor to a probability distribution, which is then used to estimate the likelihood of a true interaction (2). We validated

SAINT on multiple independent purifications for several kinases and expression levels (fig. S3 and tables S3 to S5). A final KPI dataset of 1844 interactions between 887 protein partners was generated from more than 38,000 unfiltered identifications at a stringent SAINT threshold of $P > 0.85$ (fig. S4 and tables S1 and S2). High-confidence interactions were recovered for 120 protein kinases (fig. S5; see fig. S6 and table S6 for validation). For a number of kinases, we demonstrated that associated proteins were substrates in vitro (figs. S7 and S8 and table S7). Our dataset doubled the number of KPIs obtained in previous low-throughput (LTP) studies and performed as well as LTP data against an unbiased HTP high-confidence (HTP-HC) benchmark dataset [fig. S1 (2)]. Clustering of all kinases and phosphatases by their interaction profiles revealed locally dense regions in the KPI network (Fig. 1A and fig. S9).

The Cdc14 phosphatase formed one of the largest single hubs in the network with 53 interaction partners, including 23 kinases and 5 phosphatases (Fig. 1B, fig. S6, and table S6). Cdc14 antagonizes mitotic cyclin-dependent kinase (CDK) activity and is activated by the mitotic exit network (MEN) upon completion of anaphase (3). Many Cdc14 interactors were shared with its anchor protein Net1 and the nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylase Sir2 that together with Cdc14 form the nucleolar RENT complex (4). New connections between Cdc14 and other mitotic regulators included the CDK-inhibitory kinase Swe1, the cytokinesis checkpoint protein Boi1 (5), and two activators of cytokinesis, Cbk1 and Ace2 (6). Cdc14, Net1, and Sir2 each interacted with the DNA damage checkpoint kinases Chk1 and Dun1. In support of a role for Cdc14 in the DNA damage response, we found that ectopic expression of Cdc14 caused sensitivity to the DNA-damaging agent methylmethane sulfonate (MMS), while a strain defective for Cdc14 function was sensitive to the ribonucleotide reductase inhibitor hydroxyurea (Fig. 1C). Interactions between the

¹Centre for Systems Biology, Samuel Lunenfeld Research Institute, 600 University Avenue, Toronto, Ontario, M5G 1X5, Canada.

²Department of Pathology, University of Michigan, Ann Arbor, MI 48109, USA. ³Department of Molecular Genetics, University of Toronto, 1 Kings College Circle, Toronto, Ontario, M5S 1A8, Canada. ⁴Wellcome Trust Centre for Cell Biology and School of Biological Sciences, University of Edinburgh, Mayfield Road, Edinburgh, EH9 3JR Scotland, UK.

⁵Department of Biostatistics, University of Michigan, Ann Arbor, MI 48109, USA. ⁶Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI 48109, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: gingras@lunenfeld.ca (A.C.G.), nesvi@med.umich.edu (A.I.N.), tyers@lunenfeld.ca, m.tyers@ed.ac.uk (M.T.)

RENT and the nutrient-sensing TOR complex 1 (TORC1) were supported by the finding that increased Cdc14 activity caused rapamycin sensitivity, whereas reduced Cdc14 function caused rapamycin resistance (Fig. 1D), suggesting that Cdc14 may antagonize TOR signaling. Cdc14 also interacted with the energy-sensing adenosine 5'-monophosphate (AMP)-activated kinase (AMPK) Snf1 and its upstream kinase Sak1; AMPK activates glucose-repressed genes in yeast and is an upstream inhibitor of TOR activity in metazoans (7). Deregulation of Cdc14 caused a severe defect in growth on glycerol medium and sensitivity to 2-deoxyglucose (Fig. 1D).

Cdc14 exhibited connections with three different mitogen-activated protein kinase (MAPK) modules. Interaction of the pheromone MAPK pathway kinases Fus3 and Ste7 with Cdc14 was supported by the finding that constitutive expres-

sion of Cdc14 caused partial pheromone resistance (fig. S10). Cdc14 interacted with the high osmolarity glycerol (HOG) pathway MAPK kinase Pbs2; consistently, constitutive expression of Cdc14 caused sensitivity to osmotic stress (Fig. 1E). The HOG pathway is also known to stimulate mitotic exit (8). The upstream cell wall integrity (CWI) MAPK kinase Bck1 interacted with Cdc14; a *cdc14-3* strain was sensitive to the cell wall stress agent calcofluor white (Fig. 1E). These CWI interactions extended along each pathway because the conditional MEN alleles *mob1-77* and *cdc15-2* exhibited specific synthetic lethal interactions with either *slt2Δ* or *bck1Δ* mutations; this lethality was alleviated by growth on iso-osmotic medium but not by a catalytically inactive mutant of Slf2 (Fig. 1F and fig. S10). These data reveal Cdc14 as a nexus for cell cycle, checkpoint, metabolic, and stress signals (fig. S10).

The TORC1 and TORC2 kinase complexes are conserved from yeast to human and control macromolecular synthesis and polarized morphogenesis, respectively; TORC1 is sensitive to the macrolide rapamycin, whereas TORC2 is not (9). In the KPI dataset, TORC1 and TORC2 formed a highly connected subnetwork of 28 interaction partners, including 13 kinases and 4 phosphatases (figs. S6 and S11 and table S6). These connections established new links between TORC1 and the mitochondrial retrograde (RTG) signaling pathway (10), which induces genes required for glutamate production (fig. S12). Multiple TORC1 subunits exhibited previously undocumented interactions with the kinases Fmp48, Nnk1, Npr1, and Ksp1 (Fig. 2A and fig. S11).

Fmp48 is a kinase of unknown function that is associated with mitochondrial subcellular fractions (11). Consistent with interactions among

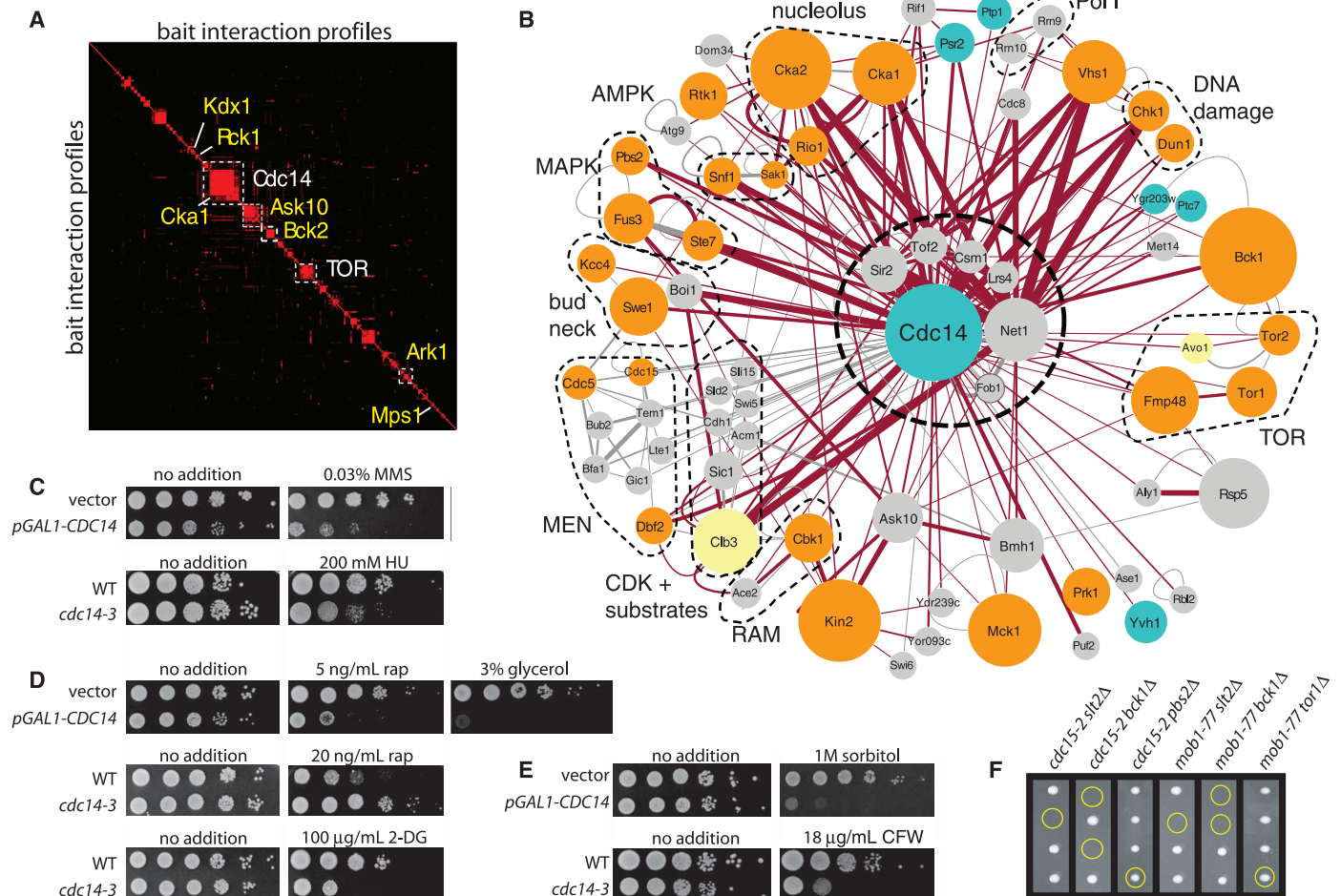


Fig. 1. Cdc14 phosphatase network. **(A)** Hierarchical two-dimensional clustering of bait interaction profiles in the KPI dataset. See fig. S9 for full clustergram. Networks for indicated clusters and other kinases are shown in fig. S19. **(B)** Cdc14-Net1-Sir2 (RENT) interaction network. Kinases are in orange, phosphatases in blue, kinase-associated proteins in yellow, and other proteins in gray. Red connecting lines indicate KPI interactions, gray lines LTP interactions, and gray dashed lines HTP-HC interactions. Line thickness indicates peptide count of interaction; node size is proportional to total number of interactions in the KPI dataset. Bold dashed circle indicates RENT complex and known associated proteins. RAM, regulation of Ace2p activity and cellular morphogenesis. **(C)** Sensitivity of a *GAL1-CDC14* strain to

0.03% methyl methanesulfonate (MMS) when induced by 0.02% galactose (see fig. S20 for expression titration) and a *cdc14-3* strain to 200 mM hydroxyurea (HU) at 33°C. **(D)** Sensitivity of a *GAL1-CDC14* strain to either rapamycin (5 ng/ml) or glycerol medium when induced by 0.05% galactose. Resistance of a *cdc14-3* strain to rapamycin (20 ng/ml) and sensitivity to 2-deoxyglucose (DG, 100 μg/ml) at 33°C. **(E)** Sensitivity of a *GAL1-CDC14* strain to 1 M sorbitol when induced by 0.05% galactose. Sensitivity of a *cdc14-3* strain to calcofluor white (CFW, 18 μg/ml) at 33°C. **(F)** Representative tetrads bearing combinations of *slt2Δ*, *bck1Δ*, *cdc15-2*, and *mob1-77* alleles. Double-mutant spore clones are circled in yellow; *pbs2Δ* and *tor1Δ* served as negative controls.

Fmp48, TORC1, and the RTG inhibitor Mks1, elevated expression of *FMP48* caused a growth defect on nonfermentable glycerol medium and rapamycin resistance on a fermentable carbon source (Fig. 2B). Overexpression of *FMP48* caused abnormal mitochondrial morphology (Fig. 2C) and repression of genes encoding tricarboxylic acid cycle enzymes, electron transport chain components, and subunits of the adenosine 5'-triphosphate (ATP) synthase (Fig. 2D). Fmp48-associated kinase activity was specifically increased by rapamycin

treatment (Fig. 2E), suggesting that Fmp48 relays TORC1 signals to the RTG pathway and mitochondrial function.

The uncharacterized kinase Ykl171w, renamed Nnk1 for nitrogen network kinase, associated with all TORC1 subunits (fig. S11) and with Gdh2, the NAD⁺-dependent glutamate dehydrogenase that catalyzes deamination of glutamate to α -ketoglutarate and ammonia (12). Gdh2 was phosphorylated by Nnk1 complexes in vitro (Fig. 2F), and a *gdh2* Δ strain was resistant to rapamycin

when grown on glutamate as the sole nitrogen source (Fig. 2G), whereas overexpression of *NNK1* conferred hypersensitivity to rapamycin (Fig. 2H). Nnk1 also interacted with the TORC1 effector Ure2, which regulates the nitrogen catabolite response by sequestering the transcription factor Gln3 in the cytoplasm (12). Overexpression of *NNK1* induced rapid nuclear accumulation of Gln3 (Fig. 2I) and increased transcription of Gln3 target genes (Fig. 2J), suggesting that Nnk1 activity antagonizes the Ure2-Gln3 inter-

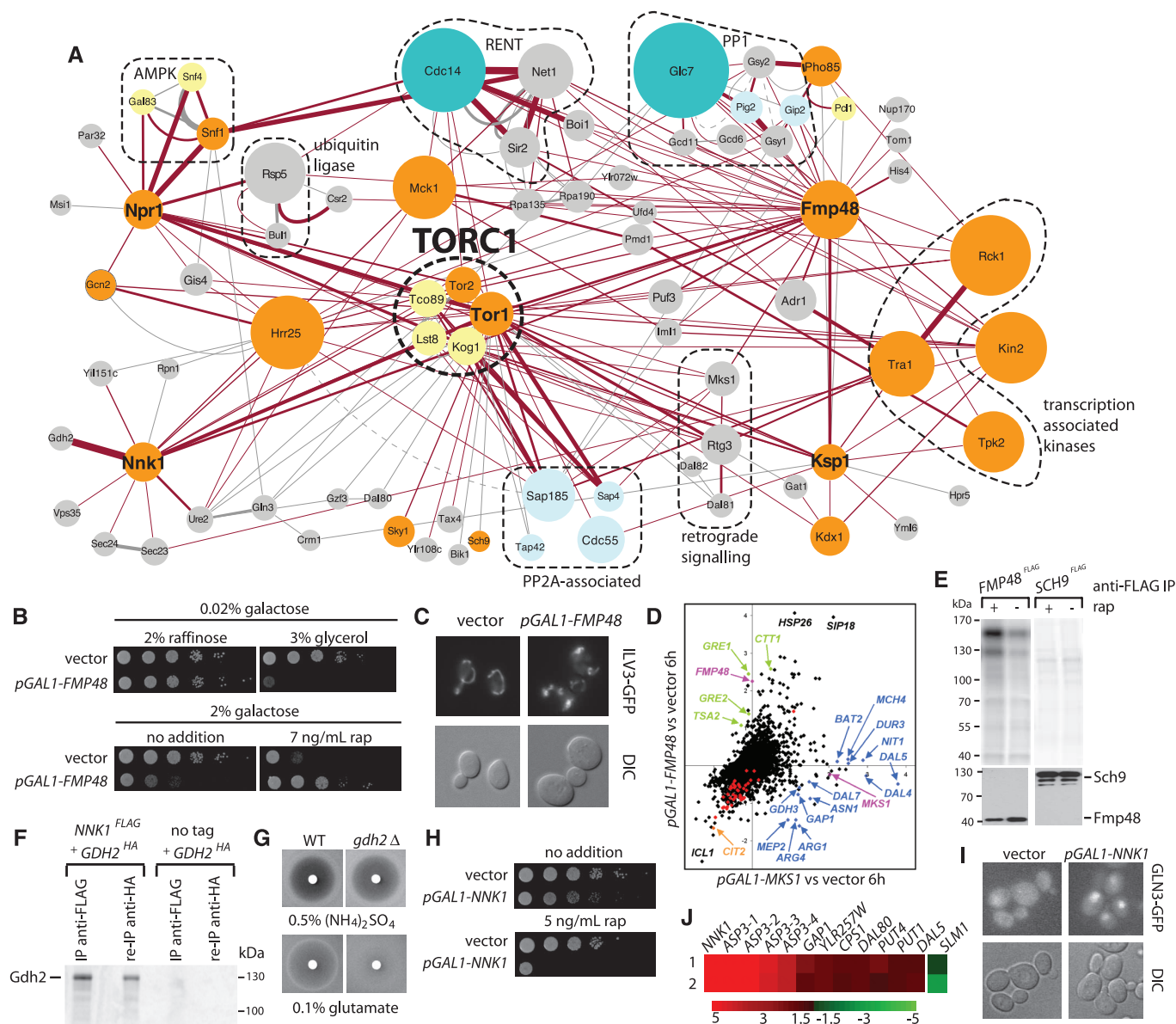


Fig. 2. TORC1 kinase network. (A) Partial network of new TORC1-associated kinases. (B) Overexpression of *GAL1-FMP48* inhibits growth on glycerol and confers rapamycin resistance. (C) Overexpression of *GAL1-FMP48* causes abnormal mitochondrial morphology as visualized with an *lv3*^{GFP} mitochondrial matrix fusion protein (GFP, green fluorescent protein). DIC, differential interference contrast. (D) Genome-wide expression profiles of *GAL1-FMP48* and *GAL1-MKS1* strains induced with 0.2% galactose. RTG-responsive (orange), mitochondrial (red), stress-responsive (green), and Gln3/Gcn4-responsive (blue) genes are marked. (E) Fmp48^{FLAG} or Sch9^{FLAG} complexes were immunoprecipitated from cells grown in the presence or absence of rapamycin (200 ng/ml) for 30 min, then incubated with

[33P]- γ -ATP, and radiolabeled species were resolved by SDS-polyacrylamide gel electrophoresis. Nonregulated Sch9-associated activity served as a negative control. (F) Immunopurified Nnk1^{FLAG} complexes were incubated with [33P]- γ -ATP, then denatured, and radiolabeled Gdh2 species were repurified with antibody to hemagglutinin (HA). (G) A *gdh2* Δ strain is rapamycin resistant when glutamate is the sole nitrogen source. (H) Expression of *GAL1-NNK1* in 2% galactose confers sensitivity to rapamycin (5 ng/ml). (I) Expression of *GAL1-NNK1* in 2% galactose for 1 hour causes nuclear accumulation of Gln3^{GFP}. (J) Expression of *GAL1-NNK1* in 0.2% galactose for 1.5 hours specifically induces Gln3 target genes. Color bar indicates fold increase (red) or decrease (green) relative to empty vector control.

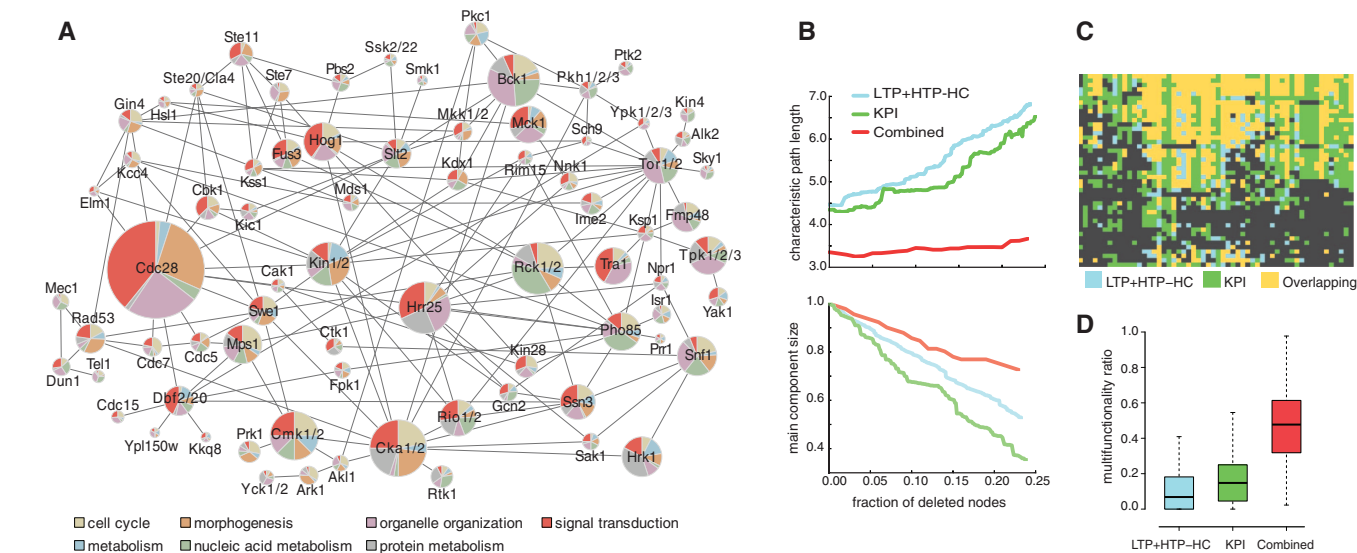


Fig. 3. A kinase-kinase (K-K) network connects the proteome. **(A)** Combined K-K interaction network derived from the KPI, LTP, and HTP-HC datasets. Interactions from known kinase regulatory subunits and paralogs were collapsed into single nodes (table S8). The reduced network contains 156 interactions between 75 kinases, 66 of which contain documented phosphorylation sites (table S9). Colors indicate fraction of GO Super-Slim biological processes assigned by interaction partners of each kinase (2). **(B)**

Nodes in the combined K-K network were deleted in decreasing degree order. Characteristic path length and largest residual connected component were normalized to initial values. K-K networks derived from KPI and LTP+HTP-HC datasets were used as controls. **(C)** Clustering of GO Slim biological processes associated with kinase interaction partners. Full clustergram is shown in fig. S17. **(D)** Multifunctionality of kinase associations. Ratio indicates number of GO Slim biological processes per kinase normalized to all processes (2).

action. The expansive TORC1 network also included other nutrient-sensing kinases (Npr1, Snf1, Gcn2, and Ksp1; fig. S11) (13), transcription-associated kinases (Tra1 and Tpk2), MAPK module components (Bck1 and Kdx1), cell cycle kinases (Ime2, Mih1, and Clb2-Cdc28), an mRNA splicing kinase (Sky1), and a ribosome biogenesis kinase (Rio2). These findings underscore the central role of TOR in cell growth.

In a global protein interaction network constructed from the KPI, LTP, and HTP-HC datasets (2), kinase-kinase (K-K) interactions were significantly enriched compared to all other kinase interaction partners ($P < 3 \times 10^{-6}$) and collectively formed a highly interconnected K-K network (Fig. 3A, figs. S13 and S14, and table S8). Consistent with a trans-kinase phosphorylation network (14), we assigned 607 phosphorylation sites on 98 kinases (fig. S15 and table S9). This K-K network was extremely robust to fragmentation by hub deletion (Fig. 3B) and was far less modular than previous less-complete K-K networks [fig. S16 (2)]. Within the global network, kinases had a significantly higher centrality compared to nonkinase nodes [$P < 10^{-16}$ (2)], suggesting that kinases might unify cellular regulation. To test this idea, we identified dense clusters of interactions (cliques or complexes) in the global interaction network, then determined the extent of clique cross-connection by kinase interactions. More than 80% of the proteome was interlinked by kinases in this manner (fig. S17), a significantly larger fraction than in random networks [$P < 10^{-8}$ (2)]. The potential for kinases to co-regulate otherwise separate functions was further revealed by the diversity of Gene Ontology (GO) biological processes associated with kinase

interaction partners (Fig. 3C and fig. S18). The multifunctionality of kinases, as defined by associated GO terms, was markedly increased by the KPI dataset (Fig. 3D).

Cellular processes are controlled by a multitude of low-affinity interactions, as often mediated by short linear motifs embedded in disordered protein regions (15, 16). The KPI network is highly enriched for disordered regions as compared to the entire proteome [$P < 10^{-16}$ (2)]. This physical organization may allow the cell to overcome stochastic limitations in signal propagation, integration, and downstream responses (16). In human cells, kinase-mediated signaling can readily propagate across pathways (17) and may dictate complex decisions through a broadly distributed network of effectors (18, 19). Moreover, phosphorylation-based feedback loops often enable cooperative responses, tuning of network outputs, and entrained states (20–22). The densely connected and non-modular architecture of the KPI network suggests that the interaction of many such circuits will underpin cellular information flow (23).

References and Notes

1. T. Pawson, *Curr. Opin. Cell Biol.* **19**, 112 (2007).
2. Supporting material is available on Science Online.
3. F. Stegmeier, A. Amon, *Annu. Rev. Genet.* **38**, 203 (2004).
4. A. F. Straight et al., *Cell* **97**, 245 (1999).
5. M. Mendoza et al., *Nat. Cell Biol.* **11**, 477 (2009).
6. B. Nelson et al., *Mol. Biol. Cell* **14**, 3782 (2003).
7. D. G. Hardie, *Nat. Rev. Mol. Cell Biol.* **8**, 774 (2007).
8. V. Reiser, K. E. D'Aquino, L. S. Ee, A. Amon, *Mol. Biol. Cell* **17**, 3136 (2006).
9. S. Wullschlegel, R. Loewith, M. N. Hall, *Cell* **124**, 471 (2006).
10. Z. Liu, R. A. Butow, *Annu. Rev. Genet.* **40**, 159 (2006).
11. J. Reinders, R. P. Zahedi, N. Pfanner, C. Meisinger, A. Sickmann, *J. Proteome Res.* **5**, 1543 (2006).
12. B. Magasanik, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 16537 (2005).

13. A. Huber et al., *Genes Dev.* **23**, 1929 (2009).
14. A. Chi et al., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 2193 (2007).
15. V. Neduvu et al., *PLoS Biol.* **3**, e405 (2005).
16. T. J. Gibson, *Trends Biochem. Sci.* **34**, 471 (2009).
17. W. M. Old et al., *Mol. Cell* **34**, 115 (2009).
18. H. Daub et al., *Mol. Cell* **31**, 438 (2008).
19. A. von Kriegsheim et al., *Nat. Cell Biol.* **11**, 1458 (2009).
20. J. E. Ferrell Jr., *Curr. Opin. Cell Biol.* **14**, 140 (2002).
21. F. Li, T. Long, Y. Lu, Q. Ouyang, C. Tang, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4781 (2004).
22. Q. A. Justman, Z. Serber, J. E. Ferrell Jr., H. El-Samad, K. M. Shokat, *Science* **324**, 509 (2009).
23. P. Nurse, *Nature* **454**, 424 (2008).
24. We thank B. Raught, A. Amon, L. Harrington, J. Bader, M. Costanzo, B. Andrews, C. Boone, R. Aebersold, B. Bodenmiller, I. Sadowski, and F. Sicheri for discussions; J. P. Zhang and D. Fermin for technical support; and M. Snyder, Y. Ohsumi, S. Piatti, S. Hahn, H. Reizman, S. Biggins, T. Petes, M. P. Longhese, and D. Mao for reagents. Supported by grants from the Canadian Institutes of Health Research to A.C.G. (MOP-84314), T.P. (MOP-57793), and M.T. (MOP-12246); the Ontario Research Fund to T.P. and A.C.G. (REO#-044); the National Institutes of Health to M.T. (R01RR024031 from the National Center for Research Resources) and A.I.N. (CA-126239); a Terry Fox Foundation Research Studentship from the National Cancer Institute of Canada to J.R.S.; Federation of European Biochemical Societies and Marie Curie Fellowships to V.N.; Canada Research Chairs in Functional Genomics and Bioinformatics (to M.T.) and in Functional Proteomics (to A.C.G.); the Lea Reichmann Chair in Cancer Proteomics to A.C.G.; and a Scottish Universities Life Sciences Alliance Research Professorship and a Royal Society Wolfson Research Merit Award to M.T.

Supporting Online Material

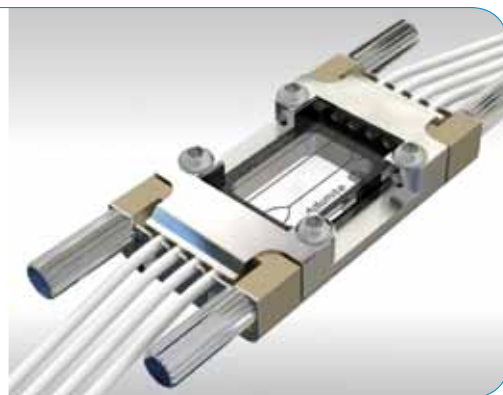
www.sciencemag.org/cgi/content/full/328/5981/1043/DC1
Materials and Methods
Figs. S1 to S27
Tables S1 to S15
References
19 May 2009; accepted 7 April 2010
10.1126/science.1176495

NEW PRODUCTS

MULTIPHASE FLOW MICROFLUIDIC CHIP

The glass microfluidic Y-Junction Chip enables liquid-liquid contact of immiscible fluid streams, as well as investigations into the diffusion of molecules between laminar flow streams. The chip has extremely smooth channel surfaces for an uninterrupted flow. With a compact size of 22.5 mm by 15 mm, the Y-Junction Chip is compatible with the Mitos Chip Holder H and the Mitos Edge Connector. Its broad temperature range, pressure range, and chemical compatibility enable a wide range of fluids to be used. Its spectral transmission facilitates high visibility, making access with microscopy systems quick and easy. It offers a range of options to tailor the chip to individual user specifications.

Dolomite

For info: +44-1763-242491 | www.dolomite-microfluidics.com

SUPER-RESOLUTION MICROSCOPES

A new family of products that allow photo-activated localization microscopy (PALM) and super-resolution structured illumination microscopy (SR-SIM) techniques to be combined enables scientists in biomedical research to image cellular structures marked with fluorescent dyes with maximum spatial resolution below the classic diffraction limit of microscopes of 200 nanometers. The line features three microscope systems. The Elyra S.1 allows the use of SR-SIM technology on the basis of a standard microscope stand. The Elyra P.1 offers PALM technology commercially for the first time, according to the manufacturer. The Elyra PS.1 combines both technologies on the same stand along with a laser-scanning microscope (LSM). This combination means that an object can be imaged with the three techniques—LSM, SR-SIM, and PALM—in succession. Elyra gives users the opportunity to acquire the super-resolution systems on either a turnkey or modular basis.

Carl Zeiss

For info: +49-(0)-3641-64-2770 | www.zeiss.de/micro-press

PROTEIN CRYSTALLOGRAPHY

The Minstrel HT UV is a fully automated, high throughput ultraviolet (UV) and visible crystal imaging and protein crystal monitoring system. Optimized for use in protein crystallization experiments, this robotic instrument is an advance over visible light microscopy technology because its UV technology can find crystals in complex drops and easily distinguish protein crystals from nonprotein crystals (such as salt or detergents). The Minstrel HT UV features Clean Light Technology, which provides illumination with the wavelength optimized for the absorption of the fluorescing amino acids, while reducing the light that can result in photo damage to proteins. The fluorescent light resulting from the protein is then digitally recorded by a specialized 5.1 megapixel charge-coupled device sensor. This camera, attached to a unique single microscope optical train, also allows for UV imaging to be combined with either monochromatic, color, or polarized color imaging in the visible spectrum.

Rigaku

For info: 760-438-5282 | www.rigaku.com

MINIATURE SPECTROMETERS

The XR Series of extended range, miniature, fiber-optic spectrometers covers a wide spectral range, providing an optical resolution of 2 nm and offering the convenience of a single, monolithic spectrometer to cover all wavelengths from 200 nm to 1,050 nm. A new XR-1 grating option overcomes the traditional challenges of providing broad ultraviolet–near-infrared coverage in a single miniature spectrometer. With a 500 lines/mm density, the grating delivers high performance at a budget-friendly price, without increasing the footprint. The XR-1 grating is available preconfigured in the USB2000+, JAZ-EL2000, and USB4000 spectrometers and can also be added as an option on custom-built systems. The instruments feature a proprietary order-sorting filter applied directly to the detector to eliminate second-order and third-order effects.

Ocean Optics

For info: 727-733-2447 | www.OceanOptics.com

MOTORIZED MICROSCOPE STAGE

The Prior H117P2IX flat-top stage for the Olympus IX series of inverted microscopes is suitable for all high-precision and material science scanning operations, with specific attention in its design given to assisting researchers doing prolonged live cell studies. The newest version of the stage maximizes access to the nosepiece for correction collar adjustment. Its miniaturized drive boxes occupy a fraction of the space of previous models, providing easy access to the nosepiece area. By mounting all the drive components below the top plate, the H117P2IX also provides easy access for micromanipulators, environmental chambers, and robotic loaders. The stage allows for scanning using a broad range of sample holders, including microtiter plates, slide holders, petri dishes, well plates, flasks, and metallurgical sample holders.

Prior Scientific

For info: 781-878-8442 | www.prior.com

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information. Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.